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(54) Title: HCV GENOMIC SEQUENCES FOR DIAGNOSTICS AND THERAPEUTICS (57) Abstract The present application features nucleic acid, peptide and antibody compositions relating to genotypes of hepatitis C virus and methods of using such compositions for diagnostic and therapeutic purposes. <div style="text-align: center; margin-top: 100px;">FILE COPY DO NOT REMOVE</div>		

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HCV GENOMIC SEQUENCES FOR
DIAGNOSTICS AND THERAPEUTICS

This application is a continuation-in-part of U.S.
5 Serial No. 07/697,326 entitled "Polynucleotide Probes
Useful for Screening for Hepatitis C Virus, filed May
8, 1991.

Technical Field

10 The invention relates to compositions and methods
for the detection and treatment of hepatitis C virus,
(HCV) infection, formerly referred to as blood-borne
non-A, non-B hepatitis virus (NANBV) infection. More
specifically, embodiments of the present invention
15 feature compositions and methods for the detection of
HCV, and for the development of vaccines for the
prophylactic treatment of infections of HCV, and
development of antibody products for conveying passive
immunity to HCV.

20

Background of the Invention

The prototype isolate of HCV was characterized in
U.S. Patent Application Serial No. 122,714 (See also
EPO Publication No. 318,216). As used herein, the term
25 "HCV" includes new isolates of the same viral species.
The term "HCV-1" referred to in U.S. Patent Application
Serial No. 122,714.

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HCV is a transmissible disease distinguishable from other forms of viral-associated liver diseases, including that caused by the known hepatitis viruses, i.e., hepatitis A virus (HAV), hepatitis B virus (HBV),
5 and delta hepatitis virus (HDV), as well as the hepatitis induced by cytomegalovirus (CMV) or Epstein-Barr virus (EBV). HCV was first identified in individuals who had received blood transfusions.

The demand for sensitive, specific methods for
10 screening and identifying carriers of HCV and HCV contaminated blood or blood products is significant. Post-transfusion hepatitis (PTH) occurs in approximately 10% of transfused patients, and HCV accounts for up to 90% of these cases. The disease
15 frequently progresses to chronic liver damage (25-55%).

Patient care as well as the prevention of transmission of HCV by blood and blood products or by close personal contact require reliable screening, diagnostic and prognostic tools to detect nucleic
20 acids, antigens and antibodies related to HCV.

Information in this application suggests the HCV has several genotypes. That is, the genetic information of the HCV virus may not be totally identical for all HCV, but encompasses groups with
25 differing genetic information.

Genetic information is stored in thread-like molecules of DNA and RNA. DNA consists of covalently

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linked chains of deoxyribonucleotides and RNA consists of covalently linked chains of ribonucleotides. Each nucleotide is characterized by one of four bases: adenine (A), guanine (G), thymine (T), and cytosine (C). The bases are complementary in the sense that, due to the orientation of functional groups, certain base pairs attract and bond to each other through hydrogen bonding and π -stacking interactions. Adenine in one strand of DNA pairs with thymine in an opposing complementary strand. Guanine in one strand of DNA pairs with cytosine in an opposing complementary strand. In RNA, the thymine base is replaced by uracil (U) which pairs with adenine in an opposing complementary strand. The genetic code of living organism is carried in the sequence of base pairs. Living cells interpret, transcribe and translate the information of nucleic acid to make proteins and peptides.

The HCV genome is comprised of a single positive strand of RNA. The HCV genome possesses a continuous, translational open reading frame (ORF) that encodes a polyprotein of about 3,000 amino acids. In the ORF, the structural protein(s) appear to be encoded in approximately the first quarter of the N-terminus region, with the majority of the polyprotein responsible for non-structural proteins.

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The HCV polyprotein comprises, from the amino terminus to the carboxy terminus, the nucleocapsid protein (C), the envelope protein (E), and the non-structural proteins (NS) 1, 2 (b), 3, 4 (b), and 5.

5 HCV of differing genotypes may encode for proteins which present an altered response to host immune systems. HCV of differing genotypes may be difficult to detect by immuno diagnostic techniques and nucleic acid probe techniques which are not specifically
10 directed to such genotype.

Definitions for selected terms used in the application are set forth below to facilitate an understanding of the invention. The term "corresponding" means homologous to or complementary to
15 a particular sequence of nucleic acid. As between nucleic acids and peptides, corresponding refers to amino acids of a peptide in an order derived from the sequence of a nucleic acid or its complement.

The term "non-naturally occurring nucleic acid"
20 refers to a portion of genomic nucleic acid, cDNA, semisynthetic nucleic acid, or synthetic origin nucleic acid which, by virtue of its origin or manipulation: (1) is not associated with all of a nucleic acid with which it is associated in nature, (2) is linked to a
25 nucleic acid or other chemical agent other than that to

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which it is linked in nature, or (3) does not occur in nature.

Similarly the term, "a non-naturally occurring peptide" refers to a portion of a large naturally occurring peptide or protein, or semi-synthetic or synthetic peptide, which by virtue of its origin or manipulation (1) is not associated with all of a peptide with which it is associated in nature, (2) is linked to peptides, functional groups or chemical agents other than that to which it is linked in nature, or (3) does not occur in nature.

The term "primer" refers to a nucleic acid which is capable of initiating the synthesis of a larger nucleic acid when placed under appropriate conditions. The primer will be completely or substantially complementary to a region of the nucleic acid to be copied. Thus, under conditions conducive to hybridization, the primer will anneal to a complementary region of a larger nucleic acid. Upon addition of suitable reactants, the primer is extended by the polymerizing agent to form a copy of the larger nucleic acid.

The term "binding pair" refers to any pair of molecules which exhibit mutual affinity or binding capacity. For the purposes of the present application, the term "ligand" will refer to one molecule of the binding pair, and the term "antiligand" or "receptor"

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or "target" will refer to the opposite molecule of the binding pair. For example, with respect to nucleic acids, a binding pair may comprise two complementary nucleic acids. One of the nucleic acids may be designated the ligand and the other strand is designated the antiligand receptor or target. The designation of ligand or antiligand is a matter of arbitrary convenience. Other binding pairs comprise, by way of example, antigens and antibodies, drugs and drug receptor sites and enzymes and enzyme substrates, to name a few.

The term "label" refers to a molecular moiety capable of detection including, by way of example, without limitation, radioactive isotopes, enzymes, luminescent agents, precipitating agents, and dyes.

The term "support" includes conventional supports such as filters and membranes as well as retrievable supports which can be substantially dispersed within a medium and removed or separated from the medium by immobilization, filtering, partitioning, or the like. The term "support means" refers to supports capable of being associated to nucleic acids, peptides or antibodies by binding partners, or covalent or noncovalent linkages.

A number of HCV strains and isolates have been identified. When compared with the sequence of the original isolate derived from the USA ("HCV-1"; see

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Q.-L. Choo et al. (1989) Science 244:359-362, Q.-L. Choo et al. (1990) Brit. Med. Bull. 46:423-441, Q.-L. Choo et al., Proc. Natl. Acad. Sci. 88:2451-2455 (1991), and E.P.O. Patent Publication No. 318,216, cited supra), it was found that a Japanese isolate ("HCV J1") differed significantly in both nucleotide and polypeptide sequence within the NS3 and NS4 regions. This conclusion was later extended to the NS5 and envelope (E1/S and E2/NS1) regions (see K. Takeuchi et al., J. Gen. Virol. (1990) 71:3027-3033, Y. Kubo, Nucl. Acids. Res. (1989) 17:10367-10372, and K. Takeuchi et al., Gene (1990) 91:287-291). The former group of isolates, originally identified in the United States, is termed "Genotype I" throughout the present disclosure, while the latter group of isolates, initially identified in Japan, is termed "Genotype II" herein.

Brief Description of the Invention

The present invention features compositions of matter comprising nucleic acids and peptides corresponding to the HCV viral genome which define different genotypes. The present invention also features methods of using the compositions corresponding to sequences of the HCV viral genome which define different genotypes described herein.

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A. Nucleic acid compositions

The nucleic acid of the present invention, corresponding to the HCV viral genome which define different genotypes, have utility as probes in nucleic acid hybridization assays, as primers for reactions involving the synthesis of nucleic acid, as binding partners for separating HCV viral nucleic acid from other constituents which may be present, and as anti-sense nucleic acid for preventing the transcription or translation of viral nucleic acid.

One embodiment of the present invention features a composition comprising a non-naturally occurring nucleic acid having a nucleic acid sequence of at least eight nucleotides corresponding to a non-HCV-1 nucleotide sequence of the hepatitis C viral genome. Preferably, the nucleotide sequence is selected from a sequence present in at least one region consisting of the NS5 region, envelope 1 region, 5'UT region, and the core region.

Preferably, with respect to sequences which correspond to the NS5 region, the sequence is selected from a sequence within a sequence numbered 2-22. The sequence numbered 1 corresponds to HCV-1. Sequences numbered 1-22 are defined in the Sequence Listing of the application.

Preferably, with respect to sequences corresponding to the envelope 1 region, the sequence is

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selected from a sequence within sequences numbered 24-32. Sequence No. 23 corresponds to HCV-1. Sequences numbered 23-32 are set forth in the Sequence Listing of the application.

5 Preferably, with respect to the sequences which correspond to the 5'UT regions, the sequence is selected from a sequence within sequences numbered 34-51. Sequence No. 33 corresponds to HCV-1. Sequence No. 33-51 are set forth in the Sequence Listing of this
10 application.

 Preferably, with respect to the sequences which correspond to the core region, the sequence is selected from a sequence within the sequences numbered 53-66. Sequence No. 52 corresponds to HCV-1. Sequences 52-66
15 are set forth in the Sequence Listing of this application.

 The compositions of the present invention form hybridization products with nucleic acid corresponding to different genotypes of HCV.

20 HCV has at least five genotypes, which will be referred to in this application by the designations GI-GV. The first genotype, GI, is exemplified by sequences numbered 1-6, 23-25, 33-38 and 52-57. The second genotype, GII, is exemplified by the sequences
25 numbered 7-12, 26-28, 39-45 and 58-64. The third genotype, GIII, is exemplified by sequences numbered 13-17, 32, 46-47 and 65-66. The fourth genotype, GIV,

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is exemplified by sequences numbered 20-22, and 29-31 and 48-49. The fifth genotype, GV, is exemplified by sequences numbered 18, 19, 50 and 51.

- One embodiment of the present invention features
- 5 compositions comprising a nucleic acid having a sequence corresponding to one or more sequences which exemplify a genotype of HCV.

B. Method of forming a Hybridization Product

- 10 Embodiments of the present invention also feature a method of forming a hybridization product with nucleic acid having a sequence corresponding to HCV nucleic acid. One method comprises the steps of
- 15 placing a non-naturally occurring nucleic acid having a non-HCV-1 sequence corresponding to HCV nucleic acid under conditions in which hybridization may occur. The non-naturally occurring nucleic acid is capable of forming a hybridization product with HCV nucleic acid, under hybridization conditions. The method further
- 20 comprises the step of imposing hybridization conditions to form a hybridization product in the presence of nucleic acid corresponding to a region of the HCV genome.

- The formation of a hybridization product has
- 25 utility for detecting the presence of one or more genotypes of HCV. Preferably, the non-naturally occurring nucleic acid forms a hybridization product

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with nucleic acid of HCV in one or more regions comprising the NS5 region, envelope 1 region, 5'UT region and the core region. To detect the hybridization product, it is useful to associate the

5 non-naturally occurring nucleic acid with a label. The formation of the hybridization product is detected by separating the hybridization product from labeled non-naturally occurring nucleic acid, which has not formed a hybridization product.

10 The formation of a hybridization product has utility as a means of separating one or more genotypes of HCV nucleic acid from other constituents potentially present. For such applications, it is useful to associate the non-naturally occurring nucleic acid with
15 a support for separating the resultant hybridization product from the the other constituents.

Nucleic acid "sandwich assays" employ one nucleic acid associated with a label and a second nucleic acid associated with a support. An embodiment of the
20 present invention features a sandwich assay comprising two nucleic acids, both have sequences which correspond to HCV nucleic acids; however, at least one non-naturally occurring nucleic acid has a sequence corresponding to non-HCV-1 HCV nucleic acid. At least
25 one nucleic acid is capable of associating with a label, and the other is capable of associating with a support. The support associated non-naturally

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occurring nucleic acid is used to separate the hybridization products which include an HCV nucleic acid and the non-naturally occurring nucleic acid having a non-HCV-1 sequence.

5 One embodiment of the present invention features a method of detecting one or more genotypes of HCV. The method comprises the steps of placing a non-naturally occurring nucleic acid under conditions which hybridization may occur. The non-naturally occurring
10 nucleic acid is capable of forming a hybridization product with nucleic acid from one or more genotypes of HCV. The first genotype, GI, is exemplified by sequences numbered 1-6, 23-25, 33-38 and 52-57. The second genotype, GII, is exemplified by the sequences
15 numbered 7-12, 26-28, 39-45 and 58-64. The third genotype, GIII, is exemplified by sequences numbered 13-17, 32, 46-47 and 65-66. The fourth genotype, GIV, is exemplified sequences numbered 20-22 and 29-31. The fifth genotype, GV, is exemplified by sequences
20 numbered 18, 19, 50 and 51.

 The hybridization product of HCV nucleic acid with a non-naturally occurring nucleic acid having non-HCV-1 sequence corresponding to sequences within the HCV genome has utility for priming a reaction for the
25 synthesis of nucleic acid.

 The hybridization product of HCV nucleic acid with a non-naturally occurring nucleic acid having a

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sequence corresponding to a particular genotype of HCV has utility for priming a reaction for the synthesis of nucleic acid of such genotype. In one embodiment, the synthesized nucleic acid is indicative of the presence
5 of one or more genotypes of HCV.

The synthesis of nucleic acid may also facilitate cloning of the nucleic acid into expression vectors which synthesize viral proteins.

Embodiments of the present methods have utility as
10 anti-sense agents for preventing the transcription or translation of viral nucleic acid. The formation of a hybridization product of a non-naturally occurring nucleic acid having sequences which correspond to a particular genotype of HCV genomic sequencing with HCV
15 nucleic acid may block translation or transcription of such genotype. Therapeutic agents can be engineered to include all five genotypes for inclusivity.

C. Peptide and antibody composition

A further embodiment of the present invention
20 features a composition of matter comprising a non-naturally occurring peptide of three or more amino acids corresponding to a nucleic acid having a non-HCV-1 sequence. Preferably, the non-HCV-1 sequence corresponds with a sequence within one or more regions
25 consisting of the NS5 region, the envelope 1 region, the 5'UT region, and the core region.

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Preferably, with respect to peptides corresponding to a nucleic acid having a non-HCV-1 sequence of the NS5 region, the sequence is within sequences numbered 2-22. The sequence numbered 1 corresponds to HCV-1. Sequences numbered 1-22 are set forth in the Sequence Listing.

Preferably, with respect to peptides corresponding to a nucleic acid having a non-HCV-1 sequence of the envelope 1 region, the sequence is within sequences numbered 24-32. The sequence numbered 23 corresponds to HCV-1. Sequences numbered 23-32 are set forth in the Sequence Listing.

Preferably, with respect to peptides corresponding to a nucleic acid having a non-HCV-1 sequence directed to the core region, the sequence is within sequences numbered 53-66. Sequence numbered 52 corresponds to HCV-1. Sequences numbered 52-66 are set forth in the Sequence Listing.

The further embodiment of the present invention features peptide compositions corresponding to nucleic acid sequences of a genotype of HCV. The first genotype, GI, is exemplified by sequences numbered 1-6, 23-25, 33-38 and 52-57. The second genotype, GII, is exemplified by the sequences numbered 7-12, 26-28, 39-45 and 58-64. The third genotype, GIII, is exemplified by sequences numbered 13-17, 32, 46-47 and 65-66. The fourth genotype, GIV, is exemplified

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sequences numbered 20-22, 29-31, 48 and 49. The fifth genotype, GV, is exemplified by sequences numbered 18, 19, 50 and 51.

5 The non-naturally occurring peptides of the present invention are useful as a component of a vaccine. The sequence information of the present invention permits the design of vaccines which are inclusive for all or some of the different genotypes of HCV. Directing a vaccine to a particular genotype
10 allows prophylactic treatment to be tailored to maximize the protection to those agents likely to be encountered. Directing a vaccine to more than one genotype allows the vaccine to be more inclusive.

The peptide compositions are also useful for the
15 development of specific antibodies to the HCV proteins. One embodiment of the present invention features as a composition of matter, an antibody to peptides corresponding to a non-HCV-1 sequence of the HCV genome. Preferably, the non-HCV-1 sequence is
20 selected from the sequence within a region consisting of the NS5 region, the envelope 1 region, and the core region. There are no peptides associated with the untranslated 5'UT region.

Preferably, with respect to antibodies directed to
25 peptides of the NS5 region, the peptide corresponds to a sequence within sequences numbered 2-22. Preferably, with respect to antibodies directed to a peptide

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corresponding to the envelope 1 region, the peptide corresponds to a sequence within sequences numbered 24-32. Preferably, with respect to the antibodies directed to peptides corresponding to the core region, the peptide corresponds to a sequence within sequences numbered 53-66.

Antibodies directed to peptides which reflect a particular genotype have utility for the detection of such genotypes of HCV and therapeutic agents.

One embodiment of the present invention features an antibody directed to a peptide corresponding to nucleic acid having sequences of a particular genotype. The first genotype, GI, is exemplified by sequences numbered 1-6, 23-25, 33-38 and 52-57. The second genotype, GII, is exemplified by the sequences numbered 7-12, 26-28, 39-45 and 58-64. The third genotype, GIII, is exemplified by sequences numbered 13-17, 32, 46-47 and 65-66. The fourth genotype, GIV, is exemplified sequences numbered 20-22, 29-31, 48 and 49. The fifth genotype, GV, is exemplified by sequences numbered 18, 19, 50 and 51.

Individuals skilled in the art will readily recognize that the compositions of the present invention can be packaged with instructions for use in the form of a kit for performing nucleic acid hybridizations or immunochemical reactions.

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The present invention is further described in the following figures which illustrate sequences demonstrating genotypes of HCV. The sequences are designated by numerals 1-145, which numerals and sequences are consistent with the numerals and sequences set forth in the Sequence Listing. Sequences 146 and 147 facilitate the discussion of an assay which numerals and sequences are consistent with the numerals and sequences set forth in the Sequence Listing.

10

Brief Description of the Figures and Sequence Listing

Figure 1 depicts schematically the genetic organization of HCV;

Figure 2 sets forth nucleic acid sequences numbered 1-22 which sequences are derived from the NS5 region of the HCV viral genome;

Figure 3 sets forth nucleic acid sequences numbered 23-32 which sequences are derived from the envelope 1 region of the HCV viral genome;

Figure 4 sets forth nucleic acid sequences numbered 33-51 which sequences are derived from the 5'UT region of the HCV viral genome; and,

Figure 5 sets forth nucleic acid sequences numbered 52-66 which sequences are derived from the core region of the HCV viral genome.

The Sequence Listing sets forth the sequences of sequences numbered 1-147.

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Detailed Description of the Invention

The present invention will be described in detail as as nucleic acid having sequences corresponding to the HCV genome and related peptides and binding
5 partners, for diagnostic and therapeutic applications.

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of chemistry, molecular biology, microbiology, recombinant DNA, and immunology, which are within the skill of the
10 art. Such techniques are explained fully in the literature. See e.g., Maniatis, Fritsch & Sambrook, Molecular Cloning; A Laboratory Manual (1982); DNA Cloning, Volumes I and II (D.N Glover ed. 1985);
15 Oligonucleotide Synthesis (M.J. Gait ed, 1984); Nucleic Acid Hybridization (B.D. Hames & S.J. Higgins eds. 1984); the series, Methods in Enzymology (Academic Press, Inc.), particularly Vol. 154 and Vol. 155 (Wu and Grossman, eds.).

The cDNA libraries are derived from nucleic acid
20 sequences present in the plasma of an HCV-infected chimpanzee. The construction of one of these libraries, the "c" library (ATCC No. 40394), is described in PCT Pub. No. WO90/14436. The sequences of the library relevant to the present invention are set
25 forth herein as sequence numbers 1, 23, 33 and 52.

Nucleic acids isolated or synthesized in accordance with features of the present invention are

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useful, by way of example without limitation as probes, primers, anti-sense genes and for developing expression systems for the synthesis of peptides corresponding to such sequences.

5 The nucleic acid sequences described define genotypes of HCV with respect to four regions of the viral genome. Figure 1 depicts schematically the organization of HCV. The four regions of particular interest are the NS5 region, the envelope 1 region, the
10 5'UT region and the core region.

 The sequences set forth in the present application as sequences numbered 1-22 suggest at least five genotypes in the NS5 region. Sequences numbered 1-22 are depicted in Figure 2 as well as the Sequence
15 Listing. Each sequence numbered 1-22 is derived from nucleic acid having 340 nucleotides from the NS5 region.

 The five genotypes are defined by groupings of the sequences defined by sequence numbered 1-22. For convenience, in the present application, the different
20 genotypes will be assigned roman numerals and the letter "G".

 The first genotype (GI) is exemplified by sequences within sequences numbered 1-6. A second genotype (GII) is exemplified by sequences within
25 sequences numbered 7-12. A third genotype (GIII) is exemplified by the sequences within sequences numbered 13-17. A fourth genotype (GIV) is exemplified by

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sequences within sequences numbered 20-22. A fifth genotype (GV) is exemplified by sequences within sequences numbered 18 and 19.

5 The sequences set forth in the present application as sequences numbered 23-32 suggest at least four genotypes in the envelope 1 region of HCV. Sequences numbered 23-32 are depicted in Figure 3 as well as in the Sequence Listing. Each sequence numbered 23-32 is derived from nucleic acid having 100 nucleotides from
10 the envelope 1 region.

A first envelope 1 genotype group (GI) is exemplified by the sequences within the sequences numbered 23-25. A second envelope 1 genotype (GII) region is exemplified by sequences within sequences
15 numbered 26-28. A third envelope 1 genotype (GIII) is exemplified by the sequences within sequences numbered 32. A fourth envelope 1 genotype (GIV) is exemplified by the sequences within sequence numbered 29-31.

The sequences set forth in the present application
20 as sequences numbered 33-51 suggest at least three genotypes in the 5'UT region of HCV. Sequences numbered 33-51 are depicted in Figure 4 as well as in the Sequence Listing. Each sequence numbered 33-51 is derived from the nucleic acid having 252 nucleotides
25 from the 5'UT region, although sequences 50 and 51 are somewhat shorter at approximately 180 nucleotides.

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The first 5'UT genotype (GI) is exemplified by the sequences within sequences numbered 33-38. A second 5'UT genotype (GII) is exemplified by the sequences within sequences numbered 39-45. A third 5'UT genotype (GIII) is exemplified by the sequences within sequences numbered 46-47. A fourth 5'UT genotype (GIV) is exemplified by sequences within sequences numbered 48 and 49. A fifth 5'UT genotype (GV) is exemplified by sequences within sequences numbered 50 and 51.

10 The sequences numbered 48-62 suggest at least three genotypes in the core region of HCV. The sequences numbered 52-66 are depicted in Figure 5 as well as in the Sequence Listing.

The first core region genotype (GI) is exemplified by the sequences within sequences numbered 52-57. The second core region genotype (GII) is exemplified by sequences within sequences numbered 58-64. The third core region genotype (GIII) is exemplified by sequences within sequences numbered 65 and 66. Sequences numbered 52-65 are comprised of 549 nucleotides. Sequence numbered 66 is comprised of 510 nucleotides.

20 The various genotypes described with respect to each region are consistent. That is, HCV having features of the first genotype with respect to the NS5 region will substantially conform to features of the first genotype of the envelope 1 region, the 5'UT region and the core region.

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Nucleic acid isolated or synthesized in accordance with the sequences set forth in sequence numbers 1-66 are useful as probes, primers, capture ligands and anti-sense agents. As probes, primers, capture ligands and anti-sense agents, the nucleic acid will normally comprise approximately eight or more nucleotides for specificity as well as the ability to form stable hybridization products.

10 Probes

A nucleic acid isolated or synthesized in accordance with a sequence defining a particular genotype of a region of the HCV genome can be used as a probe to detect such genotype or used in combination with other nucleic acid probes to detect substantially all genotypes of HCV.

With the sequence information set forth in the present application, sequences of eight or more nucleotides are identified which provide the desired inclusivity and exclusivity with respect to various genotypes within HCV, and extraneous nucleic acid sequences likely to be encountered during hybridization conditions.

Individuals skilled in the art will readily recognize that the nucleic acid sequences, for use as probes, can be provided with a label to facilitate detection of a hybridization product.

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Capture Ligand

For use as a capture ligand, the nucleic acid selected in the manner described above with respect to probes, can be readily associated with supports. The manner in which nucleic acid is associated with supports is well known. Nucleic acid having sequences corresponding to a sequence within sequences numbered 1-66 have utility to separate viral nucleic acid of one genotype from the nucleic acid of HCV of a different genotype. Nucleic acid isolated or synthesized in accordance with sequences within sequences numbered 1-66, used in combinations, have utility to capture substantially all nucleic acid of all HCV genotypes.

15 Primers

Nucleic acid isolated or synthesized in accordance with the sequences described herein have utility as primers for the amplification of HCV sequences. With respect to polymerase chain reaction (PCR) techniques, nucleic acid sequences of eight or more nucleotides corresponding to one or more sequences of sequences numbered 1-66 have utility in conjunction with suitable enzymes and reagents to create copies of the viral nucleic acid. A plurality of primers having different sequences corresponding to more than one genotype can be used to create copies of viral nucleic acid for such genotypes.

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The copies can be used in diagnostic assays to detect HCV virus. The copies can also be incorporated into cloning and expression vectors to generate polypeptides corresponding to the nucleic acid synthesized by PCR, as will be described in greater detail below.

Anti-sense

Nucleic acid isolated or synthesized in accordance with the sequences described herein have utility as anti-sense genes to prevent the expression of HCV.

Nucleic acid corresponding to a genotype of HCV is loaded into a suitable carrier such as a liposome for introduction into a cell infected with HCV. A nucleic acid having eight or more nucleotides is capable of binding to viral nucleic acid or viral messenger RNA. Preferably, the anti-sense nucleic acid is comprised of 30 or more nucleotides to provide necessary stability of a hybridization product of viral nucleic acid or viral messenger RNA. Methods for loading anti-sense nucleic acid is known in the art as exemplified by U.S. Patent 4,241,046 issued December 23, 1980 to Papahadjopoulos et al.

Peptide Synthesis

Nucleic acid isolated or synthesized in accordance with the sequences described herein have utility to

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generate peptides. The sequences exemplified by sequences numbered 1-32 and 52-66 can be cloned into suitable vectors or used to isolate nucleic acid. The isolated nucleic acid is combined with suitable DNA
5 linkers and cloned into a suitable vector. The vector can be used to transform a suitable host organism such as E. coli and the peptide encoded by the sequences isolated.

Molecular cloning techniques are described in the
10 text Molecular Cloning: A Laboratory Manual, Maniatis et al., Coldspring Harbor Laboratory (1982).

The isolated peptide has utility as an antigenic substance for the development of vaccines and antibodies directed to the particular genotype of HCV.

15

Vaccines and Antibodies

The peptide materials of the present invention have utility for the development of antibodies and vaccines.

20 The availability of cDNA sequences, or nucleotide sequences derived therefrom (including segments and modifications of the sequence), permits the construction of expression vectors encoding antigenically active regions of the peptide encoded in
25 either strand. The antigenically active regions may be derived from the NS5 region, envelope 1 regions, and the core region.

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Fragments encoding the desired peptides are derived from the cDNA clones using conventional restriction digestion or by synthetic methods, and are ligated into vectors which may, for example, contain portions of fusion sequences such as beta galactosidase or superoxide dismutase (SOD), preferably SOD. Methods and vectors which are useful for the production of polypeptides which contain fusion sequences of SOD are described in European Patent Office Publication number 0196056, published October 1, 1986.

Any desired portion of the HCV cDNA containing an open reading frame, in either sense strand, can be obtained as a recombinant peptide, such as a mature or fusion protein; alternatively, a peptide encoded in the cDNA can be provided by chemical synthesis.

The DNA encoding the desired peptide, whether in fused or mature form, and whether or not containing a signal sequence to permit secretion, may be ligated into expression vectors suitable for any convenient host. Both eukaryotic and prokaryotic host systems are presently used in forming recombinant peptides. The peptide is then isolated from lysed cells or from the culture medium and purified to the extent needed for its intended use. Purification may be by techniques known in the art, for example, differential extraction, salt fractionation, chromatography on ion exchange resins, affinity chromatography, centrifugation, and

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the like. See, for example, Methods in Enzymology for a variety of methods for purifying proteins. Such peptides can be used as diagnostics, or those which give rise to neutralizing antibodies may be formulated into vaccines. Antibodies raised against these peptides can also be used as diagnostics, or for passive immunotherapy or for isolating and identifying HCV.

10 An antigenic region of a peptide is generally relatively small--typically 8 to 10 amino acids or less in length. Fragments of as few as 5 amino acids may characterize an antigenic region. These segments may correspond to NS5 region, envelope 1 region, and the core region of the HCV genome. The 5'UT region is not
15 known to be translated. Accordingly, using the cDNAs of such regions, DNAs encoding short segments of HCV peptides corresponding to such regions can be expressed recombinantly either as fusion proteins, or as isolated peptides. In addition, short amino acid sequences can
20 be conveniently obtained by chemical synthesis. In instances wherein the synthesized peptide is correctly configured so as to provide the correct epitope, but is too small to be immunogenic, the peptide may be linked to a suitable carrier.

25 A number of techniques for obtaining such linkage are known in the art, including the formation of disulfide linkages using N-succinimidyl-3-(2-

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pyridylthio)propionate (SPDP) and succinimidyl
4-(N-maleimido-methyl)cyclohexane-1-carboxylate (SMCC)
obtained from Pierce Company, Rockford, Illinois, (if
the peptide lacks a sulfhydryl group, this can be
5 provided by addition of a cysteine residue). These
reagents create a disulfide linkage between themselves
and peptide cysteine residues on one protein and an
amide linkage through the epsilon-amino on a lysine, or
other free amino group in the other. A variety of such
10 disulfide/amide-forming agents are known. See, for
example, Immun Rev (1982) 62:185. Other bifunctional
coupling agents form a thioether rather than a
disulfide linkage. Many of these thio-ether-forming
agents are commercially available and include reactive
15 esters of 6-maleimidocaprioc acid, 2-bromoacetic acid,
2-iodoacetic acid, 4-N-maleimido-methyl)cyclohexane-1-
carboxylic acid, and the like. The carboxyl groups can
be activated by combining them with succinimide or
1-hydroxyl-2 nitro-4-sulfonic acid, sodium salt.
20 Additional methods of coupling antigens employs the
rotavirus/"binding peptide" system described in EPO
Pub. No. 259,149, the disclosure of which is
incorporated herein by reference. The foregoing list
is not meant to be exhaustive, and modifications of the
25 named compounds can clearly be used.

Any carrier may be used which does not itself
induce the production of antibodies harmful to the

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host. Suitable carriers are typically large, slowly metabolized macromolecules such as proteins; polysaccharides, such as latex functionalized Sepharose, agarose, cellulose, cellulose beads and the like; polymeric amino acids, such as polyglutamic acid, polylysine, and the like; amino acid copolymers; and inactive virus particles. Especially useful protein substrates are serum albumins, keyhole limpet hemocyanin, immunoglobulin molecules, thyroglobulin, ovalbumin, tetanus toxoid, and other proteins well known to those skilled in the art.

Peptides comprising HCV amino acid sequences encoding at least one viral epitope derived from the NS5, envelope 1, and core region are useful immunological reagents. The 5'UT region is not known to be translated. For example, peptides comprising such truncated sequences can be used as reagents in an immunoassay. These peptides also are candidate subunit antigens in compositions for antiserum production or vaccines. While the truncated sequences can be produced by various known treatments of native viral protein, it is generally preferred to make synthetic or recombinant peptides comprising HCV sequence. Peptides comprising these truncated HCV sequences can be made up entirely of HCV sequences (one or more epitopes, either contiguous or noncontiguous), or HCV sequences and heterologous sequences in a fusion protein. Useful

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heterologous sequences include sequences that provide for secretion from a recombinant host, enhance the immunological reactivity of the HCV epitope(s), or facilitate the coupling of the polypeptide to an immunoassay support or a vaccine carrier. See, E.G., EPO Pub. No. 116,201; U.S. Pat. No. 4,722,840; EPO Pub. No. 259,149; U.S. Pat. No. 4,629,783.

The size of peptides comprising the truncated HCV sequences can vary widely, the minimum size being a sequence of sufficient size to provide an HCV epitope, while the maximum size is not critical. For convenience, the maximum size usually is not substantially greater than that required to provide the desired HCV epitopes and function(s) of the heterologous sequence, if any. Typically, the truncated HCV amino acid sequence will range from about 5 to about 100 amino acids in length. More typically, however, the HCV sequence will be a maximum of about 50 amino acids in length, preferably a maximum of about 30 amino acids. It is usually desirable to select HCV sequences of at least about 10, 12 or 15 amino acids, up to a maximum of about 20 or 25 amino acids.

HCV amino acid sequences comprising epitopes can be identified in a number of ways. For example, the entire protein sequence corresponding to each of the NS5, envelope 1, and core regions can be screened by preparing a series of short peptides that together span

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the entire protein sequence of such regions. By starting with, for example, peptides of approximately 100 amino acids, it would be routine to test each peptide for the presence of epitope(s) showing a
5 desired reactivity, and then testing progressively smaller and overlapping fragments from an identified peptides of 100 amino acids to map the epitope of interest. Screening such peptides in an immunoassay is within the skill of the art. It is also known to carry
10 out a computer analysis of a protein sequence to identify potential epitopes, and then prepare peptides comprising the identified regions for screening.

The immunogenicity of the epitopes of HCV may also be enhanced by preparing them in mammalian or yeast
15 systems fused with or assembled with particle-forming proteins such as, for example, that associated with hepatitis B surface antigen. See, e.g., US 4,722,840. Constructs wherein the HCV epitope is linked directly to the particle-forming protein coding sequences
20 produce hybrids which are immunogenic with respect to the HCV epitope. In addition, all of the vectors prepared include epitopes specific to HBV, having various degrees of immunogenicity, such as, for example, the pre-S peptide. Thus, particles
25 constructed from particle forming protein which include HCV sequences are immunogenic with respect to HCV and HBV.

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Hepatitis surface antigen (HBSAg) has been shown to be formed and assembled into particles in S. cerevisiae (P. Valenzuela et al. (1982)), as well as in, for example, mammalian cells (P. Valenzuela et al. 5 1984)). The formation of such particles has been shown to enhance the immunogenicity of the monomer subunit. The constructs may also include the immunodominant epitope of HBSAg, comprising the 55 amino acids of the presurface (pre-S) region. Neurath et al. (1984). 10 Constructs of the pre-S-HBSAg particle expressible in yeast are disclosed in EPO 174,444, published March 19, 1986; hybrids including heterologous viral sequences for yeast expression are disclosed in EPO 175,261, published March 26, 1966. These constructs may also be 15 expressed in mammalian cells such as Chinese hamster ovary (CHO) cells using an SV40-dihydrofolate reductase vector (Michelle et al. (1984)).

In addition, portions of the particle-forming protein coding sequence may be replaced with codons 20 encoding an HCV epitope. In this replacement, regions which are not required to mediate the aggregation of the units to form immunogenic particles in yeast of mammals can be deleted, thus eliminating additional HBV antigenic sites from competition with the HCV epitope.

25

Vaccines

Vaccines may be prepared from one or more

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immunogenic peptides derived from HCV. The observed
homology between HCV and Flaviviruses provides
information concerning the peptides which are likely to
be most effective as vaccines, as well as the regions
5 of the genome in which they are encoded.

Multivalent vaccines against HCV may be comprised
of one or more epitopes from one or more proteins
derived from the NS5, envelope 1, and core regions. In
particular, vaccines are contemplated comprising one or
10 more HCV proteins or subunit antigens derived from the
NS5, envelope 1, and core regions. The 5'UT region is
not known to be translated.

The preparation of vaccines which contain an
immunogenic peptide as an active ingredient, is known
15 to one skilled in the art. Typically, such vaccines
are prepared as injectables, either as liquid solutions
or suspensions; solid forms suitable for solution in,
or suspension in, liquid prior to injection may also be
prepared. The preparation may also be emulsified, or
20 the protein encapsulated in liposomes. The active
immunogenic ingredients are often mixed with excipients
which are pharmaceutically acceptable and compatible
with the active ingredient. Suitable excipients are,
for example, water, saline, dextrose, glycerol,
25 ethanol, or the like and combinations thereof. In
addition, if desired, the vaccine may contain minor
amounts of auxiliary substances such as wetting or

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emulsifying agents, pH buffering agents, and/or adjuvants which enhance the effectiveness of the vaccine. Examples of adjuvants which may be effective include but are not limited to: aluminum hydroxide, N-acetyl-muramyl-L-theronyl-D- isoglutamine (thr-MDP), N-acetyl-nor-muramyl-L-alanyl- D-isoglutamine (CGP 11637, referred to as nor-MDP), N- acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1- 2-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)- ethylamine (CGP 19835A, referred to as MTP-PE), and RIBI, which contains three components extracted from bacteria, monophosphoryl lipid A, trehalose dimycolate and cell wall skeleton (MPL+TDM+CWS) in a 2% squalene/Tween 80 emulsion. The effectiveness of an adjuvant may be determined by measuring the amount of antibodies directed against an immunogenic peptide containing an HCV antigenic sequence resulting from administration of this peptide in vaccines which are also comprised of the various adjuvants.

The vaccines are conventionally administered parenterally, by injection, for example, either subcutaneously or intramuscularly. Additional formulations which are suitable for other modes of administration include suppositories and, in some cases, oral formulations. For suppositories, traditional binders and carriers may include, for example, polyalkylene glycols or triglycerides; such

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suppositories may be formed from mixtures containing the active ingredient in the range of 0/5% to 10%, preferably 1%-2%. Oral formulations include such normally employed excipients as, for example,

- 5 pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, and the like.

The examples below are provided for illustrative purposes and are not intended to limit the scope of the
10 present invention.

I. Detection of HCV RNA from Serum

- RNA was extracted from serum using guanidinium salt, phenol and chloroform according to the
15 instructions of the kit manufacturer (RNAzol™ B kit, Cinna/Biotechx). Extracted RNA was precipitated with isopropanol and washed with ethanol. A total of 25 µl serum was processed for RNA isolation, and the purified RNA was resuspended in 5 µl diethyl
20 pyrocarbonate treated water for subsequent cDNA synthesis.

II. cDNA Synthesis and Polymerase Chain Reaction (PCR) Amplification

- 25 Table 1 lists the sequence and position (with reference to HCV1) of all the PCR primers and probes used in these examples. Letter designations for

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nucleotides are consistent with 37 C.F.R. §§1.821-1.825. Thus, the letters A, C, G, T, and U are used in the ordinary sense of adenine, cytosine, guanine, thymine, and uracil. The letter M means A or C; R means A or G; W means A or T/U; S means C or G; Y means C or T/U; K means G or T/U; V means A or C or G, not T/U; H means A or C or T/U, not G; D means A or G or T/U, not C; B means C or G or T/U, not A; N means (A or C or G or T/U) or (unknown or other). Table 1 is set forth below:

Table 1		
Seq. No.	Sequence (5'-3')	Nucleotide Position
15	67 CAAACGTAACACCAACCGRCGCCCACAGG	374-402
	68 ACAGAYCCGCAKAGRTCCCCCAG	1192-1169
	69 GCAACCTCGAGGTAGACGTCAGCCTATCCC	509-538
	70 GCAACCTCGTGGAAGGCGACAACCTATCCC	509-538
	71 GTCACCAATGATTGCCCTAACTCGAGTATT	948-977
20	72 GTCACGAACGACTGCTCCAACCTCAAG	948-973
	73 TGGACATGATCGCTGGWGCYCACTGGGG	1375-1402
	74 TGGAYATGGTGGYGGGGGCYCACTGGGG	1375-1402
	75 ATGATGAACTGGTCVCCYAC	1308-1327
	76 ACCTTVGCCCAGTTSCCCRCCATGGA	1453-1428
25	77 AACCCACTCTATGYCCGGYCAT	205-226
	78 GAATCGCTGGGGTGACCG	171-188
	79 CCATGAATCACTCCCCTGTGAGGAACTA	30-57
	80 TTGCGGGGGCACGCCCAA	244-227

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For cDNA synthesis and PCR amplification, a protocol developed by Perkin-Elmer/Cetus (GeneAmp® RNA PCR kit) was used. Both random hexamer and primers with specific complementary sequences to HCV were employed to prime the reverse transcription (RT) reaction. All processes, except for adding and mixing reaction components, were performed in a thermal cycler (MJ Research, Inc.). The first strand cDNA synthesis reaction was inactivated at 99°C for 5 min, and then cooled at 50°C for 5 min before adding reaction components for subsequent amplification. After an initial 5 cycles of 97°C for 1 min, 50°C for 2 min, and 72°C for 3 min, 30 cycles of 94°C for 1 min, 55°C for 2 min, and 72°C for 3 min followed, and then a final 7 min of elongation at 72°C.

For the genotyping analysis, sequences 67 and 68 were used as primers in the PCR reaction. These primers amplify a segment corresponding to the core and envelope regions. After amplification, the reaction products were separated on an agarose gel and then transferred to a nylon membrane. The immobilized reaction products were allowed to hybridize with a ³²P-labelled nucleic acid corresponding to either Genotype I (core or envelope 1) or Genotype II (core or envelope 1). Nucleic acid corresponding to Genotype I comprised sequences numbered 69 (core), 71 (envelope), and 73 (envelope). Nucleic acid corresponding to

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Genotype II comprised sequences numbered 70 (core), 72 (envelope), and 74 (envelope).

5 The Genotype I probes only hybridized to the product amplified from isolates which had Genotype I sequence. Similarly, Genotype II probes only hybridized to the product amplified from isolates which had Genotype II sequence.

10 In another experiment, PCR products were generated using sequences 79 and 80. The products were analyzed as described above except Sequence No. 73 was used to detect Genotype I, Sequence No. 74 was used to detect Genotype II, Sequence No. 77 (5'UT) was used to detect Genotype III, and Sequence No. 78 (5'UT) was used to detect Genotype IV. Each sequence hybridized in a
15 genotype specific manner.

III. Detection of HCV GI-GIV using a sandwich hybridization assay for HCV RNA

20 An amplified solution phase nucleic acid sandwich hybridization assay format is described in this example. The assay format employs several nucleic acid probes to effect capture and detection. A capture probe nucleic acid is capable of associating a complementary probe bound to a solid support and HCV
25 nucleic acid to effect capture. A detection probe nucleic acid has a first segment (A) that binds to HCV nucleic acid and a second segment (B) that hybridizes to a second amplifier nucleic acid.

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The amplifier nucleic acid has a first segment (B*) that hybridizes to segment (B) of the probe nucleic acid and also comprises fifteen iterations of a segment (C). Segment C of the amplifier nucleic acid is
 5 capable of hybridizing to three labeled nucleic acids.

Nucleic acid sequences which correspond to nucleotide sequences of the envelope 1 gene of Group I HCV isolates are set forth in sequences numbered 81-99. Table 2 sets forth the area of the HCV genome
 10 to which the nucleic acid sequences correspond and a preferred use of the sequences.

Table 2

15	Probe Type	Sequence No.	Complement of Nucleotide Numbers
	=====		
	Label	81	879-911
	Label	82	912-944
	Capture	83	945-977
20	Label	84	978-1010
	Label	85	1011-1043
	Label	86	1044-1076
	Label	87	1077-1109
	Capture	88	1110-1142
25	Label	89	1143-1175

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Table 2 continued

	Probe Type	Sequence No.	Complement of Nucleotide Numbers
5			
	Label	90	1176-1208
	Label	91	1209-1241
	Label	92	1242-1274
	Capture	93	1275-1307
10	Label	94	1308-1340
	Label	95	1341-1373
	Label	96	1374-1406
	Label	97	1407-1439
	Capture	98	1440-1472
15	Label	99	1473-1505

Nucleic acid sequences which correspond to
 nucleotide sequences of the envelope 1 gene of Group II
 HCV isolates are set forth in sequences 100-118. Table
 20 3 sets forth the area of the HCV genome to which the
 nucleic acid corresponds and the preferred use of the
 sequences.

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Table 3

	Probe Type	Sequence No.	Complement of Nucleotide Numbers
5	=====		
	Label	100	879-911
	Label	101	912-944
	Capture	102	945-977
	Label	103	978-1010
10	Label	104	1011-1043
	Label	105	1044-1076
	Label	106	1077-1109
	Capture	107	1110-1142
	Label	108	1143-1175
15	Label	109	1176-1208
	Label	110	1209-1241
	Label	111	1242-1274
	Capture	112	1275-1307
	Label	113	1308-1340
20	Label	114	1341-1373
	Label	115	1374-1406
	Label	116	1407-1439
	Capture	117	1440-1472
	Label	118	1473-1505
25			

Nucleic acid sequences which correspond to
nucleotide sequences in the C gene and the 5'UT region

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are set forth in sequences 119-145. Table 4 identifies the sequence with a preferred use.

Table 4

	Probe Type	Sequence No.
	=====	=====
5	Capture	119
	Label	120
10	Label	121
	Label	122
	Capture	123
	Label	124
	Label	125
15	Label	126
	Capture	127
	Label	128
	Label	129
	Label	130
20	Capture	131
	Label	132
	Label	133
	Label	134
	Label	135
25	Capture	136
	Label	137
	Label	138

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Table 4 continued

	Probe Type	Sequence No.
	=====	=====
5	Label	139
	Capture	140
	Label	141
	Label	142
	Label	143
10	Capture	144
	Label	145

The detection and capture probe HCV-specific segments, and their respective names as used in this assay were as follows.

- 15 Capture sequences are sequences numbered 119-122 and 141-144.
 Detection sequences are sequences numbered 119-140.

- 20 Each detection sequence contained, in addition to the sequences substantially complementary to the HCV sequences, a 5' extension (B) which extension (B) is complementary to a segment of the second amplifier nucleic acid. The extension (B) sequence is identified in the Sequence Listing as Sequence No. 146, and is
25 reproduced below.

AGGCATAGGACCCGTGTCTT

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Each capture sequence contained, in addition to the sequences substantially complementary to HCV sequences, a sequence complementary to DNA bound to a solid phase. The sequence complementary to DNA bound to a solid support was carried downstream from the capture sequence. The sequence complementary to the DNA bound to the support is set forth as Sequence No. 147 and is reproduced below.

CTTCTTTGGAGAAAGTGGTG

10 Microtiter plates were prepared as follows. White Microlite 1 Removawell strips (polystyrene microtiter plates, 96 wells/plate) were purchased from Dynatech Inc.

15 Each well was filled with 200 μ l 1 N HCl and incubated at room temperature for 15-20 min. The plates were then washed 4 times with 1X PBS and the wells aspirated to remove liquid. The wells were then filled with 200 μ l 1 N NaOH and incubated at room temperature for 15-20 min. The plates were again washed 4 times with 1X PBS and the wells aspirated to remove liquid.

20 Poly(phe-lys) was purchased from Sigma Chemicals, Inc. This polypeptide has a 1:1 molar ratio of phe:lys and an average m.w. of 47,900 gm/mole. It has an average length of 309 amino acids and contains 155 amines/mole. A 1 mg/ml solution of the polypeptide was mixed with 2M NaCl/1X PBS to a final concentration of

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0.1 mg/ml (pH 6.0). A volume of 200 μ l of this solution was added to each well. The plate was wrapped in plastic to prevent drying and incubated at 30°C overnight. The plate was then washed 4 times with 1X
5 PBS and the wells aspirated to remove liquid.

The following procedure was used to couple the nucleic acid, a complementary sequence to Sequence No. 147, to the plates, hereinafter referred to as immobilized nucleic acid. Synthesis of immobilized
10 nucleic acid having a sequence complementary to Sequence No. 133 was described in EPA 883096976. A quantity of 20 mg disuccinimidyl suberate was dissolved in 300 μ l dimethyl formamide (DMF). A quantity of 26
15 OD₂₆₀ units of immobilized nucleic acid was added to 100 μ l coupling buffer (50 mM sodium phosphate, pH 7.8). The coupling mixture was then added to the DSS-DMF solution and stirred with a magnetic stirrer for 30 min. An NAP-25 column was equilibrated with 10
20 mM sodium phosphate, pH 6.5. The coupling mixture DSS-DMF solution was added to 2 ml 10 mM sodium phosphate, pH 6.5, at 4°C. The mixture was vortexed to mix and loaded onto the equilibrated NAP-25 column. DSS-activated immobilized nucleic acid DNA was eluted from the column with 3.5 ml 10 mM sodium phosphate, pH
25 6.5. A quantity of 5.6 OD₂₆₀ units of eluted DSS-activated immobilized nucleic acid DNA was added to 1500 ml 50 mM sodium phosphate, pH 7.8. A volume of 50

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µl of this solution was added to each well and the plates were incubated overnight. The plate was then washed 4 times with 1X PBS and the wells aspirated to remove liquid.

- 5 Final stripping of plates was accomplished as follows. A volume of 200 µl of 0.2N NaOH containing 0.5% (w/v) SDS was added to each well. The plate was wrapped in plastic and incubated at 65°C for 60 min. The plate was then washed 4 times with 1X PBS and the
10 wells aspirated to remove liquid. The stripped plate was stored with desiccant beads at 2-8°C.

Serum samples to be assayed were analyzed using PCR followed by sequence analysis to determine the genotype.

- 15 Sample preparation consisted of delivering 50 µl of the serum sample and 150 µl P-K Buffer (2 mg/ml proteinase K in 53 mM Tris-HCl, pH 8.0/0.6 M NaCl/0.06 M sodium citrate/8 mM EDTA, pH 8.0/1.3%SDS/16µg/ml
20 sonicated salmon sperm DNA/7% formamide/50 fmoles capture probes/160 fmoles detection probes) to each well. Plates were agitated to mix the contents in the well, covered and incubated for 16 hr at 62°C.

- After a further 10 minute period at room temperature, the contents of each well were aspirated
25 to remove all fluid, and the wells washed 2X with washing buffer (0.1% SDS/0.015 M NaCl/ 0.0015 M sodium citrate). The amplifier nucleic acid was then added to

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each well (50 μ l of 0.7 fmole/ μ l solution in 0.48 M NaCl/0.048 M sodium citrate/0.1% SDS/0.5% "blocking reagent" (Boehringer Mannheim, catalog No. 1096 176)). After covering the plates and agitating to mix the contents in the wells, the plates were incubated for 30 min. at 52°C.

After a further 10 min period at room temperature, the wells were washed as described above.

Alkaline phosphatase label nucleic acid, disclosed in EP 883096976, was then added to each well (50 μ l/well of 2.66 fmoles/ μ l). After incubation at 52°C for 15 min., and 10 min. at room temperature, the wells were washed twice as above and then 3X with 0.015 M NaCl/0.0015 M sodium citrate.

An enzyme-triggered dioxetane (Schaap et al., Tet. Lett. (1987) 28:1159-1162 and EPA Pub. No. 0254051), obtained from Lumigen, Inc., was employed. A quantity of 50 μ l Lumiphos 530 (Lumigen) was added to each well. The wells were tapped lightly so that the reagent would fall to the bottom and gently swirled to distribute the reagent evenly over the bottom. The wells were covered and incubated at 37°C for 20-40 min.

Plates were then read on a Dynatech ML 1000 luminometer. Output was given as the full integral of the light produced during the reaction.

The assay positively detected each of the serum samples, regardless of genotype.

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IV. Expression of the Polypeptide Encoded in Sequences Defined by Differing Genotypes

HCV polypeptides encoded by a sequence within sequences 1-66 are expressed as a fusion polypeptide with superoxide dismutase (SOD). A cDNA carrying such sequences is subcloned into the expression vector pSODcfl (Steimer et al. 1986)).

First, DNA isolated from pSODcfl is treated with BamHI and EcoRI, and the following linker was ligated into the linear DNA created by the restriction enzymes:

5 GAT CCT GGA ATT CTG ATA AGA
CCT TAA GAC TAT TTT AA 3

After cloning, the plasmid containing the insert is isolated.

Plasmid containing the insert is restricted with EcoRI. The HCV cDNA is ligated into this EcoRI linearized plasmid DNA. The DNA mixture is used to transform E. coli strain D1210 (Sadler et al. (1980)). Polypeptides are isolated on gels.

V. Antigenicity of Polypeptides

The antigenicity of polypeptides formed in Section IV is evaluated in the following manner. Polyethylene pins arranged on a block in an 8 12 array (Coselco Mimetopes, Victoria, Australia) are prepared by placing the pins in a bath (20% v/v piperidine in dimethylformamide (DMF)) for 30 minutes at room

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temperature. The pins are removed, washed in DMF for 5 minutes, then washed in methanol four times (2 min/wash). The pins are allowed to air dry for at least 10 minutes, then washed a final time in DMF (5Min). 1-Hydroxybenzotriazole (HOBt, 367 mg) is dissolved in DMF (80 μ L) for use in coupling Fmoc-protected polypeptides prepared in Section IV.

The protected amino acids are placed in micro-titer plate wells with HOBt, and the pin block placed over the plate, immersing the pins in the wells. The assembly is then sealed in a plastic bag and allowed to react at 25°C for 18 hours to couple the first amino acids to the pins. The block is then removed, and the pins washed with DMF (2 min.), MeOH (4 x, 2 min.), and again with DMF (2 min.) to clean and deprotect the bound amino acids. The procedure is repeated for each additional amino acid coupled, until all octamers are prepared.

The free N-termini are then acetylated to compensate for the free amide, as most of the epitopes are not found at the N-terminus and thus would not have the associated positive charge. Acetylation is accomplished by filling the wells of a microtiter plate with DMF/acetic anhydride/triethylamine (5:2:1 v/v/v) and allowing the pins to react in the wells for 90 minutes at 20°C. The pins are then washed with DMF (2

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min.) and MeOH (4 x, 2 min.), and air dried for at least 10 minutes.

5 The side chain protecting groups are removed by treating the pins with trifluoroacetic acid/phenol/dithioethane (95:2.5:1.5, v/v/v) in polypropylene bags for 4 hours at room temperature. The pins are then washed in dichloromethane (2 x, 2 min.), 5% di-isopropylethylamine/dichloromethane (2 x, 5 min.), 10 dichloromethane (5 min.), and air-dried for at least 10 minutes. The pins are then washed in water (2 min.), MeOH (18 hours), dried in vacuo, and stored in sealed plastic bags over silica gel. IV.B.15.b Assay of Peptides.

15 Octamer-bearing pins are treated by sonicating for 30 minutes in a disruption buffer (1% sodium dodecylsulfate, 0.1% 2-mercaptoethanol, 0.1 M NaH₂PO₄) at 60°C. The pins are then immersed several times in water (60°C), followed by boiling MeOH (2 min.), and allowed to air dry.

20 The pins are then precoated for 1 hour at 25°C in microtiter wells containing 200 µL blocking buffer (1% ovalbumin, 1% BSA, 0.1% Tween, and 0.05% NaN₃ in PBS), with agitation. The pins are then immersed in microtiter wells containing 175 µL antisera obtained 25 from human patients diagnosed as having HCV and allowed to incubate at 4°C overnight. The formation of a complex between polyclonal antibodies of the serum and

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the polypeptide initiates that the peptides give rise to an immune response in vivo. Such peptides are candidates for the development of vaccines.

Thus, this invention has been described and
5 illustrated. It will be apparent to those skilled in the art that many variations and modifications can be made without departing from the purview of the appended claims and without departing from the teaching and scope of the present invention.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

- 5 (i) APPLICANT: Tai-An Cha
- (ii) TITLE OF INVENTION: HCV GENOMIC SEQUENCES
 FOR DIAGNOSTICS AND THERAPEUTICS
- 10 (iii) NUMBER OF SEQUENCES: 147
- (iv) CORRESPONDENCE ADDRESS:
- (A) ADDRESSEE: Wolf, Greenfield & Sacks, P.C.
- (B) STREET: 600 Atlantic Avenue
- 15 (C) CITY: Boston
- (D) STATE: Massachusetts
- (E) COUNTRY: USA
- (F) ZIP: 02210
- 20 (v) COMPUTER READABLE FORM:
- (A) MEDIUM TYPE: Diskette, 5.25 inch
- (B) COMPUTER: IBM compatible
- (C) OPERATING SYSTEM: MS-DOS Version 3.3
- (D) SOFTWARE: WordPerfect 5.1

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TCTCTATCTT CCTCTTGGCT CTGCTGTCC

549

(2) INFORMATION FOR SEQ ID NO: 60

5 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 549 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

10

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

- (C) INDIVIDUAL ISOLATE: nac5

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 60

	ATGAGCACAA ATCCTAAACC CCAAAGAAAA ACCAAACGTA	40
	ACACCAACCG TCGCCACAG GACGTCAAGT TCCCGGGCGG	80
	TGGTCAGATC GTTGGTGGAG TTTACCTGTT GCCGCGCAGG	120
20	GGCCCCAGGT TGGGTGTGCG CGCGACTAGG AAGACTTCCG	160
	AGCGGTCGCA ACCTCGTGGA AGGCGACAAC CTATCCCCAA	200
	GGCTCGCCGG CCCGAGGGCA GGTCTGGGC TCAGCCCGGG	240
	TACCCTTGGC CCTCTATGG CAACGAGGGT ATGGGGTGGG	280
	CAGGATGGCT CCTGTACCC CGCGGCTCCC GGCCTAGTTG	320
25	GGGCCCCACG GACCCCCGGC GTAGGTCGCG TAATTTGGGT	360
	AAGGTCATCG ATACCCTCAC ATGCGGCTTC GCCGACCTCA	400

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TGGGGTACAT TCCGCTCGTC GCGCCCCCCC TAGGGGGCGC 440
TGCCAGGGCC CTGGCACATG GTGTCCGGGT TCTGGAGGAC 480
GGCGTGA ACT ATGCAACAGG GAATTTGCCT GGTGCTCTT 520
TCTCTATCTT CCTCTTGGCT CTGCTGTCC 549

5

(2) INFORMATION FOR SEQ ID NO: 61

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 549 nucleotides
(B) TYPE: nucleic acid
10 (C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

15 (vi) ORIGINAL SOURCE:

(C) INDIVIDUAL ISOLATE: arg2

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 61

ATGAGCACGA ATCCTAAACC TCAAAGAAAA ACCAAACGTA 40
20 ACACCAACCG CCGCCACAG GACGTCAAGT TCCCGGGCGG 80
TGGTCAGATC GTTGGTGGAG TTTACTTGTT GCCGCGCAGG 120
GGCCCCAGGT TGGGTGTGCG CGCGACTAGG AAGACTTCCG 160
AGCGGTCGCA ACCTCGTGGA AGGCGACAAC CTATCCCCAA 200
GGCTCGCCAG CCCGAGGGTA GGGCCTGGGC TCAGCCCCGG 240
25 TACCCTTGGC CCCTCTATGG CAATGAGGGT ATGGGGTGGG 280
CAGGGTGGCT CCTGTCCCCC CGCGGCTCCC GGCCTAGTTG 320

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(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 145

5 CARRAGGAAG AKAGAGAAAG AGCAACCRGG MAR 33

(2) INFORMATION FOR SEQ ID NO: 146

(i) SEQUENCE CHARACTERISTICS:

10 (A) LENGTH: 20 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 146

AGGCATAGGA CCCGTGTCTT 20

20 (2) INFORMATION FOR SEQ ID NO: 147

(i) SEQUENCE CHARACTERISTICS:

25 (A) LENGTH: 20 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 147

CTTCTTTGGA GAAAGTGGTG 20

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CLAIMS

1. As a composition of matter, a non-naturally occurring nucleic acid having a non-HCV-1 nucleotide
5 sequence of eight or more nucleotides corresponding to a nucleotide sequence within the hepatitis C virus genome.
2. The composition of claim 1 wherein said nucleotide
10 sequence corresponding to a non-HCV-1 nucleotide sequence within the hepatitis C virus genome is selected from the regions consisting of the NS5 region, envelope 1 region, 5'UT region, and the core region.
- 15 3. The composition of claim 1 wherein said nucleotide sequence corresponding to a non-HCV-1 nucleotide sequence within the hepatitis C virus genome corresponds to a sequence in the NS5 region.
- 20 4. The composition of claim 3 wherein said nucleotide sequence corresponding to a non-HCV-1 sequence within the hepatitis C virus genome is selected from a sequence within sequences numbered 2-22.

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5. The composition of claim 1 wherein said nucleotide sequence corresponding to a non-HCV-1 nucleotide sequence within the hepatitis C virus genome corresponds to a sequence in the envelope 1 region.

5

6. The composition of claim 5 wherein said nucleotide sequence corresponding to a non-HCV-1 sequence within the hepatitis C virus genome corresponds to a sequence within sequence numbers 24-32.

10

7. The composition of claim 1 wherein at least one sequence corresponding to a non-HCV-1 nucleotide sequence within the hepatitis C virus genome corresponds to a sequence in the 5'UT region.

15

8. The composition of claim 7 wherein said nucleotide sequence corresponding to a non-HCV-1 sequence within the hepatitis C virus genome corresponds to a sequence within sequences numbered 34-51.

20

9. The composition of claim 1 wherein said nucleotide sequence corresponding to a non-HCV-1 nucleotide sequence within the hepatitis C virus genome corresponds to a sequence in the core region.

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10. The composition of claim 9 wherein said nucleotide sequence corresponding to a non-HCV-1 sequence within the hepatitis C virus genome corresponds to a within sequences numbered 53-66.

5

11. The composition of claim 1 wherein said non-naturally occurring nucleic acid has a nucleotide sequence corresponding to one or more genotypes of hepatitis C virus.

10

12. The composition of claim 11 wherein said non-naturally occurring nucleic acid has a sequence corresponding to a sequence of a first genotype which first genotype is defined substantially by sequences numbered 1-6 in the NS5 region, 23-25 in the envelope 1 region, 33-38 in the 5'UT region, and 52-57 in the core region.

13. The composition of claim 11 wherein said non-naturally occurring nucleic acid has a sequence corresponding to a sequence of a second genotype which second genotype is defined substantially by sequences numbered 7-12 in the NS5 region, 26-28 in the envelope 1 region, 39-45 in the 5'UT region, and 58-64 in the core region.

25

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14. The composition of claim 11 wherein said non-naturally occurring nucleic acid has a sequence corresponding to a sequence of a third genotype which third genotype is defined substantially by sequences
5 numbered 13-17 in the NS5 region, 32 in the envelope 1 region, 46-47 in the 5'UT region and 65-66 in the core region.

15. The composition of claim 11 wherein said
10 non-naturally occurring nucleic acid has a sequence corresponding to a sequence of a fourth genotype which fourth genotype is defined substantially by sequences numbered 20-22 in the NS5 region, 29-31 in the envelope 1 region and 48-49 in the 5'UT region.

15
16. The composition of claim 11 wherein said non-naturally occurring nucleic acid has a sequence corresponding to a sequence of a fifth genotype which fifth genotype is defined substantially by sequences
20 numbered 18-19 in the NS5 region and 50-51 in the 5'UT region.

17. The composition of claim 1 wherein said non-naturally occurring nucleic acid is capable of
25 priming a reaction for the synthesis of nucleic acid to form a nucleic acid having a nucleotide sequence corresponding to hepatitis C virus.

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18. The composition of claim 1 wherein said non-naturally occurring nucleic acid has label means for detecting a hybridization product.
- 5 19. The composition of claim 1 wherein said non-naturally occurring nucleic acid has support means for separating a hybridization product from solution.
- 10 20. The composition of claim 1 wherein said non-naturally occurring nucleic acid prevents the transcription or translation of viral nucleic acid.
- 15 21. A method of forming a hybridization product with a hepatitis C virus nucleic acid comprising the following steps:
- 20 a. placing a non-naturally occurring nucleic acid having a nucleotide sequence of eight or more nucleotides corresponding to a non-HCV-1 sequence in the hepatitis C viral genome into conditions in which hybridization conditions can be imposed said non-naturally occurring nucleic acid capable of forming a hybridization product with said hepatitis C virus nucleic acid under hybridization
- 25 conditions; and

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b. imposing hybridization conditions to form a hybridization product in the presence of hepatitis C virus nucleic acid.

5 22. The method of claim 21 wherein said nucleotide
sequence corresponding to a non-HCV-1 sequence in the
hepatitis C virus genome corresponds to a sequence
within at least one of the regions consisting
essentially of NS5 region, envelope 1 region, 5'UT
10 region, and the core region.

23. The method of claim 21 wherein said nucleotide
sequence corresponds to a non-HCV-1 sequence
corresponds to a sequence within the NS5 region.

15

24. The method of claim 23 wherein said nucleotide
sequence corresponds to a non-HCV-1 sequence
corresponds to a sequence within sequences numbered
2-22.

20

25. The method of claim 21 wherein said nucleotide
sequence corresponds to a non-HCV-1 sequence
corresponds to a sequence within the envelope 1 region.

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26. The method of claim 25 wherein said nucleotide sequence corresponds to a non-HCV-1 sequence is selected from a sequence within sequences numbered 24-32.

5

27. The method of claim 21 wherein said nucleotide sequence corresponds to a non-HCV-1 sequence corresponding to a sequence within the 5'UT region.

10 28. The method of claim 27 wherein said nucleotide sequence corresponds to a non-HCV-1 sequence selected from a sequence within sequences numbered 34-51.

15 29. The method of claim 21 wherein said nucleotide sequence corresponds to a non-HCV-1 sequence corresponding to a sequence within the core region.

20 30. The method of claim 29 wherein said nucleotide sequence corresponds to a non-HCV-1 sequence selected from a sequence within sequences numbered 53-66.

25 31. The method of claim 21 wherein said nucleotide sequence corresponds to a non-HCV-1 nucleotide sequence corresponding to one or more genotypes of hepatitis C virus.

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32. The method of claim 21 wherein said non-naturally occurring nucleic acid has a sequence corresponding to a sequence of a first genotype which first genotype is defined substantially by sequences numbered 1-6 in the NS5 region, 23-25 in the envelope 1 region, 33-38 in the 5'UT region, and 52-57 in the core region.

33. The method of claim 21 wherein said non-naturally occurring nucleic acid has a sequence corresponding to a sequence of a second genotype which second genotype is defined substantially by sequences numbered 7-12 in the NS5 region, 26-28 in the envelope 1 region, 39-45 in the 5'UT region, and 58-64 in the core region.

34. The method of claim 21 wherein said non-naturally occurring nucleic acid has a sequence corresponding to a sequence of a third genotype which third genotype is defined substantially by sequences numbered 13-17 in the NS5 region, 32 in the envelope 1 region, 46-47 in the 5'UT region and 65-66 in the core region.

35. The method of claim 21 wherein said non-naturally occurring nucleic acid has a sequence corresponding to a sequence of a fourth genotype which fourth genotype is defined substantially by sequences numbered 20-22 in the NS5 region, 29-31 in the envelope 1 region and 48-49 in the 5'UT region.

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36. The method of claim 21 wherein said non-naturally occurring nucleic acid has a sequence corresponding to a sequence of a fifth genotype which fifth genotype is defined substantially by sequences numbered 18-19 in the NS5 region and 50-51 in the 5'UT region.

5

37. The method of claim 21 wherein said hybridization product is capable of priming a reaction for the synthesis of nucleic acid.

10

38. The method of claim 21 wherein said non-naturally occurring nucleic acid has label means for detecting a hybridization product.

39. The method of claim 21 wherein said non-naturally occurring nucleic acid has support means for separating the hybridization product from solution.

15

40. The method of claim 21 wherein said non-naturally occurring nucleic acid prevents the transcription or translation of viral nucleic acid.

20

41. As a composition of matter, a non-naturally occurring polypeptide corresponding to a non-HCV-1 nucleotide sequence of nine or more nucleotides which sequence of nine or more nucleotides corresponds to a sequence within hepatitis C virus genomic sequences.

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42. The composition of claim 41 wherein said non-HCV-1 sequence is selected from one of the regions consisting of NS5 region, envelope 1 region, and the core region.
- 5 43. The composition of claim 41 wherein said non-HCV-1 nucleotide sequence corresponds to a sequence in the NS5 region.
- 10 44. The composition of claim 43 wherein said non-HCV-1 sequence is selected from a sequence within sequences numbered 2-22.
- 15 45. The composition of claim 41 wherein said non-HCV-1 sequence corresponds to a sequence in the envelope 1 region.
- 20 46. The composition of claim 45 wherein said non-HCV-1 sequence is selected from a sequence within sequences numbered 24-32.
- 25 47. The composition of claim 41 wherein said non-HCV-1 sequence corresponds to a sequence in the core region.
48. The composition of claim 47 wherein said non-HCV-1 sequence is selected from a sequence within sequences numbered 52-66.

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49. The composition of claim 41 wherein said non-HCV-1 nucleotide sequence has a nucleotide sequence corresponding to one or more genotypes of hepatitis C virus.

5

50. The composition of claim 41 wherein said non-HCV-1 nucleotide sequence has a sequence corresponding to a sequence of a first genotype which first genotype is defined substantially by sequences numbered 1-6 in the
10 NS5 region, 23-25 in the envelope 1 region, and 52-57 in the core region.

51. The composition of claim 41 wherein said non-HCV-1 nucleotide sequence has a sequence corresponding to a
15 sequence of a second genotype which second genotype is defined substantially by sequences numbered 7-12 in the NS5 region, 26-28 in the envelope 1 region, and 58-64 in the core region.

20 52. The composition of claim 41 wherein said non-HCV-1 nucleotide sequence has a sequence corresponding to a sequence of a third genotype which third genotype is defined substantially by sequences numbered 13-17 in the NS5 region, 32 in the envelope 1 region, and 65-66
25 in the core region.

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53. The composition of claim 41 wherein said non-HCV-1 nucleotide sequence has a sequence corresponding to a sequence of a fourth genotype which fourth genotype is defined substantially by sequences numbered 20-22 in the NS5 region, 29-31 in the envelope 1 region and 48-49 in the 5'UT region.

54. The composition of claim 41 wherein said non-HCV-1 nucleotide sequence has a sequence corresponding to a sequence of a fifth genotype which fifth genotype is defined substantially by sequences numbered 18-19 in the NS5 region and 50-51 in the 5'UT region.

55. The composition of claim 41 wherein said polypeptide is capable of generating an immune reaction in a host.

56. An antibody capable of selectively binding to the composition of claim 41.

20

57. A method of detecting one or more genotypes of hepatitis C virus comprising the following steps:

a) placing a non-naturally occurring nucleic acid having a nucleotide sequence of eight or more nucleotides corresponding to one or more genotypes of hepatitis C virus under conditions where hybridization conditions can be imposed,

25

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- b) imposing hybridization conditions to form a hybridization product in the presence of hepatitis C virus nucleic acid; and
- 5 c) monitoring the non-naturally occurring nucleic acid for the formation of a hybridization product, which hybridization product is indicative of the presence of the genotype of hepatitis C virus.

10 58. The method of claim 57 wherein said non-naturally occurring nucleic acid has a sequence corresponding to a sequence of a first genotype which first genotype is defined substantially by sequences numbered 1-6 in the NS5 region, 23-25 in the envelope 1 region, 33-38 in the 5'UT region, and 52-57 in the core region.

15

59. The method of claim 57 wherein said non-naturally occurring nucleic acid has a sequence corresponding to a sequence of a second genotype which second genotype is defined substantially by sequences numbered 7-12 in the NS5 region, 26-28 in the envelope 1 region, 39-45

20 in the 5'UT region, and 58-64 in the core region.

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60. The method of claim 57 wherein said non-naturally occurring nucleic acid has a sequence corresponding to a sequence of a third genotype which third genotype is defined substantially by sequences numbered 13-17 in the NS5 region, 32 in the envelope 1 region, 46-47 in the 5'UT region and 65-66 in the core region.

61. The method of claim 57 wherein said non-naturally occurring nucleic acid has a sequence corresponding to a sequence of a fourth genotype which fourth genotype is defined substantially by sequences numbered 20-22 in the NS5 region, 29-31 in the envelope 1 region and 48-49 in the 5'UT region.

62. The method of claim 57 wherein said non-naturally occurring nucleic acid has a sequence corresponding to a sequence of a fifth genotype which fifth genotype is defined substantially by sequences numbered 18-19 in the NS5 region.

63. The method of claim 57 wherein said non-naturally occurring nucleic acid has a sequence corresponding to a sequence numbered 67-145.

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64. The method of claim 57 wherein said non-naturally occurring nucleic acid has a sequence corresponding to a sequence numbered 69, 71, 73 and 81-99 to identify Group I genotypes in the core and region of the HCV genome.

65. The method of claim 57 wherein said non-naturally occurring nucleic acid has a sequence corresponding to a sequence numbered 70, 72, 70 and 100-118 to identify Group II genotypes in the core and envelope regions of the HCV genome.

66. The method of claim 57 wherein said non-naturally occurring nucleic acid has a sequence corresponding to a sequence numbered 77 to identify Group III genotypes in the 5' UT region of the HCV genome.

67. The method of claim 57 wherein said non-naturally occurring nucleic acid has a sequence numbered 79 to identify Group IV genotypes in the 5' UT region of the HCV genome.

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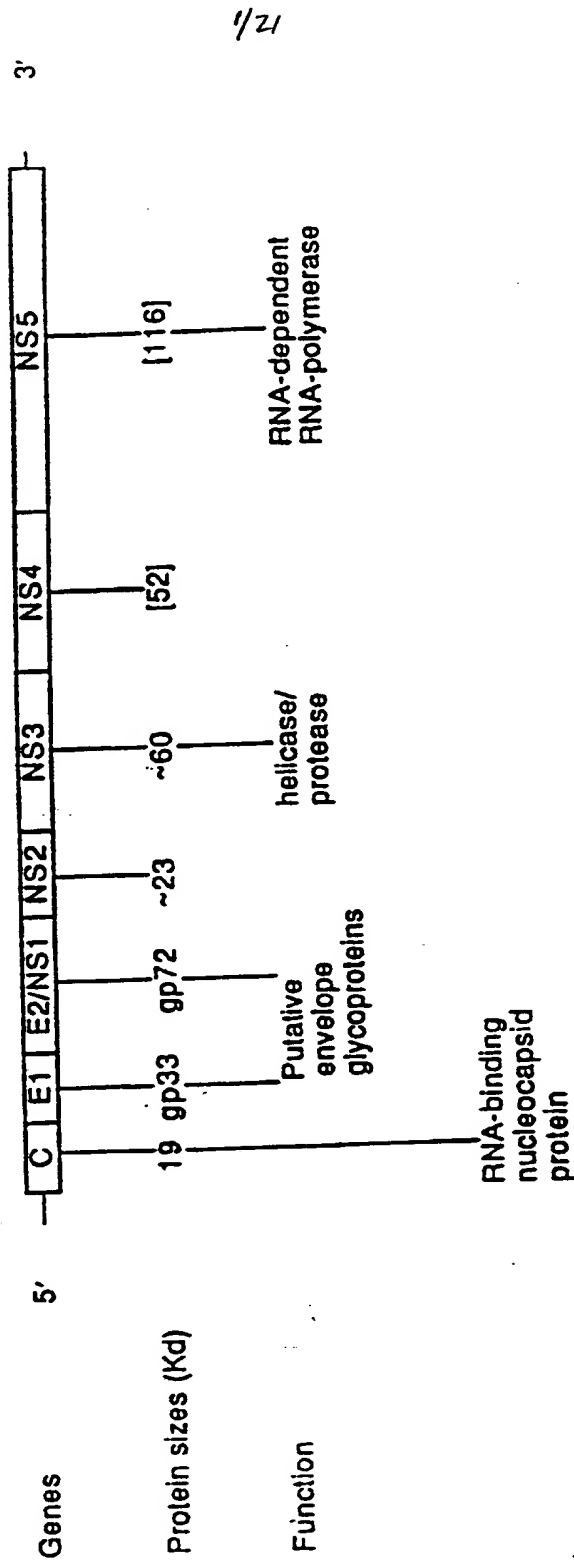


Fig. 1

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Fig. 2a

NS5 REGION

SEQUENCE	ID NUMBER	GENOTYPE	
=====			
1	GI	1	CTCCACAGTC ACTGAGAGCG ACATCCGTAC GGAGGAGGCA ATCTACCAAT GTTGTGACCT CGACCCCCAA
2		1	CTCCACAGTC ACTGAGAGCG ACATCCGTAC GGAGGAGGCA ATCTACCAAT GTTGTGACCT GGACCCCCAA
3		1	CTCCACAGTC ACTGAGAGCG ACATCCGTAC GGAGGAGGCA ATCTACCAAT GTTGTGATCT GGACCCCCAA
4		1	CTCTACAGTC ACTGAGAACG ACATCCGTAC GGAGGAGGCA ATTTACCAAT GTTGTGACCT GGACCCCCAA
5		1	CTCCACAGTC ACTGAGAGCG ATATCCGTAC GGAGGAGGCA ATCTACCAAT GTTGTGACCT GGACCCCCAA
6		1	CTCTACAGTC ACTGAGAGCG ATATCCGTAC GGAGGAGGCA ATCTACCAAT GTTGTGACCT GGACCCCCAA
7	GII	1	CTCCACAGTC ACTGAGAGTG ACATCCGTGT TGAGGAGTCA ATTTACCAAT GTTGTGACCT GGCCCCCGAA
8		1	CTCAACGGTC ACTGAGAGTG ACATCCGTGT TGAGGAGTCA ATTTACCAAT GTTGTGACCT GGCCCCCGAG
9		1	CTCAACGGTC ACCGAGAGTG ACATCCGTGT TGAGGAGTCA ATTTACCAAT GTTGTGACCT GGCCCCCGAG
10		1	CTCAACGGTC ACTGAGAGTG ACATCCGTGT CGAGGAGTCC ATTTACCAAT GTTGTGACCT GGCCCCCGAA
11		1	CTCCACAGTC ACTGAGAGTG ACATCCGTGT TGAGGAGTCA ATTTACCAAT GTTGTGACCT GGCCCCCGAA
12		1	CTCAACAGTC ACTGAGAGTG ACATCCGTGT TGAGGAGTCA ATCTACCAAT GTTGTGACCT GGCCCCCGAA
13	GIII	1	CTCAACCGTC ACTGAGAGG ACATCAGAAC TGAGGAGTCC ATATACCGAG CCTGCTCCCT GCCTGAGGAG
14		1	CTCTACAGTC ACCTAAAGG ACATCAGATC CTAGGAGTCC ATCTACCAAT CCTGTTTACT GCCCGAGGAG
15		1	CTCTACAGTC ACAGAGAGGG ACATCAGAAC CGAGGAGTCC ATCTATCTGT CCTGTTTACT GCCTGAGGAG
16		1	CTCTACAGTC ACGGAGAGGG ACATCAGAAC CGAGGAGTCC ATCTATCTGT CCTGTTTACT GCCTGAGGAG
17		1	CTCAACCGTC ACGGAGAGGG ACATCAGAAC AGAAGATCC ATATATCAGG GTTGTTCCT GCCTCAGGAG
18	GV	1	CTCGACCGTT ACCGAACATG ACATATGAC TGAAGAGTCT ATTTACCAAT CATTTACTT GCAGCCTGAG
19		1	CTCGACCGTT ACCGAACATG ACATATGAC TGAAGAGTCC ATTTACCAAT CATTTACTT GCAGCCTGAG
20	GIV	1	CTCTACTGTC ACTGAACAGG ACATCAGGTT GGAAGAGGAG ATATACCAAT GCTGTAACTT TGAACCGGAG
21		1	CTCGACTGTC ACTGAACAGG ACATCAGGTT GGAAGAGGAG ATATACCAAT GCTGTAACTT TGAACCGGAG
22		1	CTCAACTGTC ACTGAACAGG ACATCAGGTT GGAAGAGGAG ATATACCAAT GCTGTAACTT TGAACCGGAG
=====			

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Fig. 2b

NS5 REGION - (2/5)

SEQUENCE			+++++		=====	
SEQUENCE	ID NUMBER	GENOTYPE				
1	71	GI	GCCCGCGTGG	CCATCAAGTC	CCTCACCGAG	AGGCTTTATG TTGGGGGCCC TCTTACCAAT TCAAGGGGGG
2	71	GI	GCCCGCATGG	CCATCAAGTC	CCTCACTGAG	AGGCTTTATG TCGGGGGCCC TCTTACCAAT TCAAGGGGGG
3	71	GI	GCCCGCGTGG	CCATCAAGTC	CCTCACTGAG	AGGCTTTATG TTGGGGGCCC TCTTACCAAT TCAAGGGGGG
4	71	GI	GCCCGCGTGG	CCATCAAGTC	CCTCACTGAG	AGGCTTTATG TTGGGGGCCC TCTTACCAAT TCAAGGGGGG
5	71	GI	GCCCGCGTGG	CCATCAAGTC	CCTCACCGAG	AGGCTTTATG TCGGGGGCCC TCTTACCAAT TCAAGGGGGG
6	71	GI	GCCCGTGTGG	CCATCAAGTC	CCTCACTGAG	AGGCTTTATG TTGGGGGCCC TCTTACCAAT TCAAGGGGGG
7	71	GI	GCCAGACAGG	CCATAAGGTC	GCTCACAGAG	CGGCTCTATG TCGGGGGTCC TATGACTAAC TCCAAAGGGC
8	71	GI	GCCAGACAAG	CCATAAGGTC	GCTCACAGAG	CGGCTTTTACA TCGGGGGCCC CCTGACTAAT TCAAAAGGGC
9	71	GI	GCTAGACAGG	CCATAAGGTC	GCTCACAGAG	CGGCTTTTATA TCGGGGGCCC CCTGACTAAT TCAAAAGGGC
10	71	GI	GCCAGGCAGG	CCATAAGGTC	GCTCACCGAG	CGACTTTTATA TCGGGGGCCC CCTGACTAAT TCAAAAGGGC
11	71	GI	GCCAGACAGG	CTATAAGGTC	GCTCACAGAG	CGGCTGTACA TCGGGGGTCC CCTGACTAAT TCAAAAGGGC
12	71	GI	GCCAGACAGG	CTATAAGGTC	GCTCACAGAG	CGGCTTTTACA TCGGGGGTCC CCTGACTAAT TCAAAAGGGC
13	71	GI	GCTCACATTG	CCATACACTC	GCTGACTGAG	AGGCTCTACG TGGGAGGGCC CATGTTCAAC AGCAAGGGCC
14	71	GI	GCTCGAACTG	CTATACACTC	ACTGACTGAG	AGACTATACG TAGGGGGGCC CATGACAAAC AGCAAGGGCC
15	71	GI	GCCGGAAGTG	CTATACACTC	ACTGACTGAG	AGACTGTACG TAGGGGGGCC CATGACAAAC AGCAAGGGCC
16	71	GI	GCTCGAACTG	CCATACACTC	ACTGACTGAG	AGGCTGTACG TAGGGGGGCC CATGACAAAC AGCAAGGGCC
17	71	GI	GCTAGAACTG	CTATCCACTC	GCTCACTGAG	AGACTCTACG TAGGAGGGCC CATGACAAAC AGCAAGGGAC
18	71	GV	GCGCGTGTGG	CAATACGGTC	ACTCACCAA	CGCCTGTACT GTGGAGGGCC CATGTATAAC AGCAAGGGCC
19	71	GV	GCACCGCGGG	CAATACGGTC	ACTCACCAA	CGCCTGTACT GTGGAGGGCC CATGTATAAC AGCAAGGGCC
20	71	GI	GCCAGGAAAG	TGATCTCCTC	CCTCACGGAG	CGGCTTTACT GCGGGGGCCC TATGTTCAAC AGCAAGGGGG
21	71	GI	GCCAGGAAAG	TGATCTCCTC	CCTCACGGAG	CGGCTTTACT GCGGGGGCCC TATGTTCAAT AGCAAGGGGG
22	71	GI	GCCAGGAAAG	TGATCTCCTC	CCTCACGGAA	CGGCTTTACT GCGGGGGCCC TATGTTCAAC AGCAAGGGGG

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Fig. 2c

NS5 REGION - (3/5)

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SEQUENCE ID NUMBER	GENOTYPE	
1	GI	141 AGAACTGCGG CTATCGCAGG TGCCGCGCGA GCGGCGTACT GACAACTAGC TGTGGTAACA CCCTCACTTG
2		141 AGAACTGCGG CTATCGCAGG TGCCGCGCGA GCGGCGTACT GACAACTAGC TGTGGTAACA CCCTCACTTG
3		141 AGAACTGCGG CTATCGCAGG TGCCGCGCGA GCGGCGTACT GACAACTAGC TGTGGTAACA CCCTCACTTG
4		141 AAAAATGCGG CTATCGCAGG TGCCGCGCGA GCGGCGTACT GACAACTAGC TGTGGTAACA CCCTCACTTG
5		141 AAAAATGCGG CTATCGCAGG TGCCGCGCGA GCGGCGTACT GACAACTAGC TGTGGTAACA CCCTCACTTG
6		141 AGAACTGCGG CTATCGCAGG TGCCGCGCGA GCGGCGTACT GACAACTAGC TGTGGTAACA CCCTCACTTG
7	GII	141 AGAACTGCGG CTATCGCAGG TGCCGCGCGA GCGGCGTACT GACAACTAGC TGTGGTAACA CCCTCACTTG
8		141 AGAACTGCGG CTATCGCAGG TGCCGCGCGA GCGGCGTACT GACAACTAGC TGTGGTAACA CCCTCACTTG
9		141 AGAACTGCGG CTATCGCAGG TGCCGCGCGA GCGGCGTACT GACAACTAGC TGTGGTAACA CCCTCACTTG
10		141 AGAACTGCGG CTATCGCAGG TGCCGCGCGA GCGGCGTACT GACAACTAGC TGTGGTAACA CCCTCACTTG
11		141 AGAACTGCGG CTATCGCAGG TGCCGCGCGA GCGGCGTACT GACAACTAGC TGTGGTAACA CCCTCACTTG
12		141 AGAACTGCGG CTATCGCAGG TGCCGCGCGA GCGGCGTACT GACAACTAGC TGTGGTAACA CCCTCACTTG
13	GIII	141 AGAACTGCGG CTATCGCAGG TGCCGCGCGA GCGGCGTACT GACAACTAGC TGTGGTAACA CCCTCACTTG
14		141 AATCCTGCGG GTACAGGCGT TGCCGCGCGA GCGGCGTACT GACAACTAGC TGTGGTAACA CCCTCACTTG
15		141 AATCCTGCGG GTACAGGCGT TGCCGCGCGA GCGGCGTACT GACAACTAGC TGTGGTAACA CCCTCACTTG
16		141 AATCCTGCGG GTACAGGCGT TGCCGCGCGA GCGGCGTACT GACAACTAGC TGTGGTAACA CCCTCACTTG
17		141 AATCCTGCGG GTACAGGCGT TGCCGCGCGA GCGGCGTACT GACAACTAGC TGTGGTAACA CCCTCACTTG
18	GV	141 AACAAATGCG GTATCGTAGA TGCCGCGCGA GCGGCGTACT GACAACTAGC TGTGGTAACA CCCTCACTTG
19		141 AACAAATGCG GTATCGTAGA TGCCGCGCGA GCGGCGTACT GACAACTAGC TGTGGTAACA CCCTCACTTG
20	GIV	141 CCCAGTGTGG TTATCGCGGT TGCCGCGCGA GCGGCGTACT GACAACTAGC TGTGGTAACA CCCTCACTTG
21		141 CCCAGTGTGG TTATCGCGGT TGCCGCGCGA GCGGCGTACT GACAACTAGC TGTGGTAACA CCCTCACTTG
22		141 CCCAGTGTGG TTATCGCGGT TGCCGCGCGA GCGGCGTACT GACAACTAGC TGTGGTAACA CCCTCACTTG

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NS5 REGION - (4/5)

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NS5 REGION - (5/5)

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Fig. 3

ENVELOPE REGION

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SEQUENCE	ID NUMBER	GENOTYPE
23	1	GACGGCGTTG GTAATGGCTC AGCTGCTCCG GATCCACAA GCCATCTTGG ACATGATCGC
24	1	GACGGCGTTG GTGGTAGCTC AGGTACTCCG GATCCACAA GCCATCATGG ACATGATCGC
25	1	AACGGCGCTG GTAGTAGCTC AGCTGCTCAG GGTCGGCAA GCCATCGTGG ACATGATCGC
26	1	GACAGCCCTA GTGGTATCGC AGTTACTCCG GATCCACAA GCCGTCATGG ATATGGTGGC
27	1	AGCAGCCCTA GTGGTATCGC AGTTACTCCG GATCCACAA AGCATCGTGG ACATGGTGGC
28	1	GACAGCCCTA GTGGTATCGC AGTTACTCCG GATCCACAA GCTGTCTGGG ACATGGTGGC
29	1	TGTGGGTATG GTGGTGGCGC ACGTCCTGCG TTGCCCCAG ACCTTGTTTCG ACATAATAGC
30	1	TGTGGGTATG GTGGTAGCAC ACGTCCTGCG TCTGCCCCAG ACCTTGTTTCG ACATAATAGC
31	1	TGTGGGTATG GTGGTGGCGC AAGTCCTGCG TTGCCCCAG ACCTTGTTTCG ACGTGTCTAGC
32	1	TACCACTATG CTCCTGGCAT ACTTGTGGCG CATCCGGGAG GTCATCCCTGG ACATTATCAC

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23	61	TGGTGCTCAC TGGGGAGTCC TGGCGGGCAT AGCGTATTTC
24	61	TGGAGCCAC TGGGGAGTCC TGGCGGGCAT AGCGTATTTC
25	61	TGGTGCCAC TGGGGAGTCC TAGCGGGCAT AGCGTATTTC
26	61	GGGGCCAC TGGGGAGTCC TGGCGGGCAT TGCCTACTAT
27	61	GGGGCCAC TGGGGAGTCC TGGCGGGCAT TGCCTACTAT
28	61	GGGGCCAC TGGGGAATCC TAGCGGGTCT TGCCTACTAT
29	61	CGGGCCAT TGGGGCATCT TGGCGGGCAT GGCCTATTAC
30	61	CGGGCCAT TGGGGCATCT TGGCAGGCCT AGCCTATTAC
31	61	CGGGCCAT TGGGGCATCT TGGCGGGCAT GGCCTATTAC
32	61	GGGAGGAC TGGGGCGTGA TGTGTGGCCT GGCCTATTTC

=====

100 Total

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Fig. 4a

5'UT Region

SEQUENCE	ID NUMBER	GENOTYPE	
=====			=====
33	1	GI	GTTAGTATGA GTGTCGTGCA GCCTCCAGGA CCCCCCTCC CGGGAGAGCC ATAGTGGTCT
34	1		GTTAGTATGA GTGTCGTGCA GCCTCCAGGA CCCCCCTCC CGGGAGAGCC ATAGTGGTCT
35	1		GTTAGTATGA GTGTCGTGCA GCCTCCAGGA CCCCCCTCC CGGGAGAGCC ATAGTGGTCT
36	1		GTTAGTATGA GTGTCGTGCA GCCTCCAGGA CCCCCCTCC CGGGAGAGCC ATAGTGGTCT
37	1		GTTAGTATGA GTGTCGTGCA GCCTCCAGGA CCCCCCTCC CGGGAGAGCC ATAGTGGTCT
38	1		GTTAGTATGA GTGTCGTGCA GCCTCCAGGA CCCCCCTCC CGGGAGAGCC ATAGTGGTCT
=====			=====
39	1	GII	GTTAGTATGA GTGTCGTGCA GCCTCCAGGA CCCCCCTCC CGGGAGAGCC ATAGTGGTCT
40	1		GTTAGTATGA GTGTCGTGCA GCCTCCAGGA CCCCCCTCC CGGGAGAGCC ATAGTGGTCT
41	1		GTTAGTATGA GTGTCGTGCA GCCTCCAGGA CCCCCCTCC CGGGAGAGCC ATAGTGGTCT
42	1		GTTAGTATGA GTGTCGTGCA GCCTCCAGGA CCCCCCTCC CGGGAGAGCC ATAGTGGTCT
43	1		GTTAGTATGA GTGTCGTGCA GCCTCCAGGA CCCCCCTCC CGGGAGAGCC ATAGTGGTCT
44	1		GTTAGTATGA GTGTCGTGCA GCCTCCAGGA CCCCCCTCC CGGGAGAGCC ATAGTGGTCT
45	1		GTTAGTATGA GTGTCGTGCA GCCTCCAGGA CCCCCCTCC CGGGAGAGCC ATAGTGGTCT
=====			=====
46	1	GIII	GCTAGTATCA GTGTCGTGCA GCCTCCAGG CCCCCCTCC CGGGAGAGCC ATAGTGGTCT
47	1		GTTAGTATGA GTGTCGTGCA GCCTCCAGG CCCCCCTCC CGGGAGAGCC ATAGTGGTCT
=====			=====
48	1	GIV	GTTAGTACGA GTGTCGTGCA GCCTCCAGGA CTCCTCCCTCC CGGGAGAGCC ATAGTGGTCT
49	1		GTTAGTACGA GTGTCGTGCA GCCTCCAGGA CTCCTCCCTCC CGGGAGAGCC ATAGTGGTCT
=====			=====
50	1	GV	GTTAGTATGA GTGTCGAACA GCCTCCAGGA CCCCCCTCC CGGGAGAGCC ATAGTGGTCT
51	1		GTTAGTATGA GTGTCGAACA GCCTCCAGGA CCCCCCTCC CGGGAGAGCC ATAGTGGTCT
=====			=====

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Fig. 4b

5'UT Region (2/5)

SEQUENCE			GENOTYPE			
ID NUMBER						
33	GC	GA	ACCG	TTAG	TACACC	GGAATTGCCA
34	GC	GA	ACCG	TTAG	TACACC	GGAATTGCCA
35	GC	GA	ACCG	TTAG	TACACC	GGAATTGCCA
36	GC	GA	ACCG	TTAG	TACACC	GGAATTGCCA
37	GC	GA	ACCG	TTAG	TACACC	GGAATTGCCA
38	GC	GA	ACCG	TTAG	TACACC	GGAATTGCCA
39	GC	GA	ACCG	TTAG	TACACC	GGAATTGCCA
40	GC	GA	ACCG	TTAG	TACACC	GGAATTGCCA
41	GC	GA	ACCG	TTAG	TACACC	GGAATTGCCA
42	GC	GA	ACCG	TTAG	TACACC	GGAATTGCCA
43	GC	GA	ACCG	TTAG	TACACC	GGAATTGCCA
44	GC	GA	ACCG	TTAG	TACACC	GGAATTGCCA
45	GC	GA	ACCG	TTAG	TACACC	GGAATTGCCA
46	GC	GA	ACCG	TTAG	TACACC	GGAATTGCCA
47	GC	GA	ACCG	TTAG	TACACC	GGAATTGCCA
48	GC	GA	ACCG	TTAG	TACACC	GGAATTGCCA
49	GC	GA	ACCG	TTAG	TACACC	GGAATTGCCA
50	GC	GA	ACCG	TTAG	TACACC	GGAATTGCCA
51	GC	GA	ACCG	TTAG	TACACC	GGAATTGCCA

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Fig. 4c

5'UT Region (3/5)

```
=====
SEQUENCE
ID NUMBER  GENOTYPE
=====
33      121  G1      TGGAGATTG GCGTGCCCC CGAAGACTG CTAGCCGAGT AGTGTGGGT
34      121  G1      TGGAGATTG GCGTGCCCC CGAAGACTG CTAGCCGAGT AGTGTGGGT
35      121  G1      TGGAGATTG GCGTGCCCC CGAAGACTG CTAGCCGAGT AGTGTGGGT
36      121  G1      TGGAGATTG GCGTGCCCC CGAAGACTG CTAGCCGAGT AGTGTGGGT
37      121  G1      TGGAGATTG GCGTGCCCC CGAAGACTG CTAGCCGAGT AGTGTGGGT
38      121  G1      TGGAGATTG GCGTGCCCC CGAAGACTG CTAGCCGAGT AGTGTGGGT
=====
39      121  GII     TGGAGATTG GCGTGCCCC CGGAGACTG CTAGCCGAGT AGTGTGGGT
40      121  GII     TGGAGATTG GCGTGCCCC CGGAGACTG CTAGCCGAGT AGTGTGGGT
41      121  GII     TGGAGATTG GCGTGCCCC CGGAGACTG CTAGCCGAGT AGTGTGGGT
42      121  GII     TGGAGATTG GCGTGCCCC CGGAGACTG CTAGCCGAGT AGTGTGGGT
43      121  GII     TGGAGATTG GCGTGCCCC CGGAGACTG CTAGCCGAGT AGTGTGGGT
44      121  GII     TGGAGATTG GCGTGCCCC CGGAGACTG CTAGCCGAGT AGTGTGGGT
45      121  GII     TGGAGATTG GCGTGCCCC CGGAGACTG CTAGCCGAGT AGTGTGGGT
=====
46      121  GIII    CAGCCATTG GCGTGCCCC CGAAGACTG CTAGCCGAGT AGCGTTGGGT
47      121  GIII    CAGCCATTG GCGTGCCCC CGAAGACTG CTAGCCGAGT AGCGTTGGGT
=====
48      121  GIV     CAGAAATTG GCGTGCCCC CGGAGACTG CTAGCCGAGT AGTGTGGGT
49      121  GIV     CAGAAATTG GCGTGCCCC CGGAGACTG CTAGCCGAGT AGTGTGGGT
=====
50      121  GV      CCGAGATTG GCGTGCCCC CGGAGACTG CTAGCCGAGT AGTGTGGGT
51      121  GV      CCGAGATTG GCGTGCCCC CGGAGACTG CTAGCCGAGT AGTGTGGGT
=====
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Fig. 4d

ENVELOPE REGION (4/5)

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=====
SEQUENCE
ID NUMBER  GENOTYPE
=====
33      181      G1      CGCGAAAGGC CTTGTGGTAC TGCCTGATAG GGTGCTTGCG AGTGCCCCCG GAGGTCTCGT
34      181      CGCGAAAGGC CTTGTGGTAC TGCCTGATAG GGTGCTTGCG AGTGCCCCCG GAGGTCTCGT
35      181      CGCGAAAGGC CTTGTGGTAC TGCCTGATAG GGTGCTTGCG AGTGCCCCCG GAGGTCTCGT
36      181      CGCGAAAGGC CTTGTGGTAC TGCCTGATAG GGTGCTTGCG AGTGCCCCCG GAGGTCTCGT
37      181      CGCGAAAGGC CTTGTGGTAC TGCCTGATAG GGTGCTTGCG AGTGCCCCCG GAGGTCTCGT
38      181      CGCGAAAGGC CTTGTGGTAC TGCCTGATAG GGTGCTTGCG AGTGCCCCCG GAGGTCTCGT
=====
39      181      G11     CGCGAAAGGC CTTGTGGTAC TGCCTGATAG GGTGCTTGCG AGTGCCCCCG GAGGTCTCGT
40      181      CGCGAAAGGC CTTGTGGTAC TGCCTGATAG GGTGCTTGCG AGTGCCCCCG GAGGTCTCGT
41      181      CGCGAAAGGC CTTGTGGTAC TGCCTGATAG GGTGCTTGCG AGTGCCCCCG GAGGTCTCGT
42      181      CGCGAAAGGC CTTGTGGTAC TGCCTGATAG GGTGCTTGCG AGTGCCCCCG GAGGTCTCGT
43      181      CGCGAAAGGC CTTGTGGTAC TGCCTGATAG GGTGCTTGCG AGTGCCCCCG GAGGTCTCGT
44      181      CGCGAAAGGC CTTGTGGTAC TGCCTGATAG GGTGCTTGCG AGTGCCCCCG GAGGTCTCGT
45      181      CGCGAAAGGC CTTGTGGTAC TGCCTGATAG GGTGCTTGCG AGTGCCCCCG GAGGTCTCGT
=====
46      181      G11     TCGGAAAGGC CTTGTGGTAC TGCCTGATAG GGTGCTTGCG AGTGCCCCCG GAGGTCTCGT
47      181      TCGGAAAGGC CTTGTGGTAC TGCCTGATAG GGTGCTTGCG AGTGCCCCCG GAGGTCTCGT
=====
48      181      G1V     CGCGAAAGGC CTTGTGGTAC TGCCTGATAG GGTGCTTGCG AGTGCCCCCG GAGGTCTCGT
49      181      CGCGAAAGGC CTTGTGGTAC TGCCTGATAG GGTGCTTGCG AGTGCCCCCG GAGGTCTCGT
=====
```

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Fig. 4e

5'UT Region (5/5)

SEQUENCE		
ID NUMBER	GENOTYPE	
33	GI	241 AGACCGTGCA CC
34		241 AGACCGTGCA CC
35		241 AGACCGTGCA CC
36		241 AGACCGTGCA CC
37		241 AGACCGTGCA CC
38		241 AGACCGTGCA CC
39	GII	241 AGACCGTGCA CC
40		241 AGACCGTGCA TC
41		241 AGACCGTGCA CC
42		241 AGACCGTGCA CC
43		241 AGACCGTGCA CC
44		241 AGACCGTGCA CC
45		241 AGACCGTGCA CC
46	GIII	241 AGACCGTGCA TC
47		241 AGACCGTGCA TC
48	GIV	241 AGACCGTGCA AC
49		241 AGACCGTGCA AC
252 Total		

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Fig. 5a

CORE REGION

SEQUENCE	ID NUMBER	GENOTYPE	SEQUENCE
52	GI	1	ATGAGCACGA ATCCTAAACC TCAAGAGAAA AACAAACGTA ACACCAACCG TCGCCCCACAG
53		1	ATGAGCACGA ATCCTAAACC TCAAGAGAAA ACCAAACGTA ACACCAACCG TCGCCCCACAG
54		1	ATGAGCACGA ATCCTAAACC TCAAGAGAAA ACCAAACGTA ACACCAACCG TCGCCCCACAG
55		1	ATGAGCACGA ATCCTAAACC TCAAGAGAAA ACCAAACGTA ACACCAACCG TCGCCCCACAG
56		1	ATGAGCACGA ATCCTAAACC TCAAGAGAAA ACCAAACGTA ACACCAACCG TCGCCCCACAG
57		1	ATGAGCACGA ATCCTAAACC TCAAGAGAAA ACCAAACGTA ACACCAACCG TCGCCCCACAG
58	GII	1	ATGAGCACGA ATCCTAAACC TCAAGAGAAA ACCAAACGTA ACACCAACCG CCGCCCCACAG
59		1	ATGAGCACAA ATCCTAAACC TCAAGAGAAA ACCAAACGTA ACACCAACCG CCGCCCCACAG
60		1	ATGAGCACAA ATCCTAAACC CCAAGAGAAA ACCAAACGTA ACACCAACCG TCGCCCCACAG
61		1	ATGAGCACGA ATCCTAAACC TCAAGAGAAA ACCAAACGTA ACACCAACCG CCGCCCCACAG
62		1	ATGAGCACGA ATCCTAAACC TCAAGAGAAA ACCAAACGTA ACACCAACCG CCGCCCCACAG
63		1	ATGAGCACGA ATCCTAAACC TCAAGAGAAA ACCAAACGTA ACACCAACCG CCGCCCCACAG
64		1	ATGAGCACGA ATCCTAAACC TCAAGAGAAA ACCAAACGTA ACACCAACCG CCGCCCCACAG
65	GIII	1	ATGAGCACAA ATCCTAAACC TCAAGAGAAA ACCAAAGAA AACTAACCG CCGCCCCACAG
66		1	ATGAGCACAA ATCGTCAACC TCAAGAGAAA ACCAAAGAA AACTAACCG CCGCCCCACAG

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Fig. 5b

CORE REGION (2/9)

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=====
SEQUENCE
ID NUMBER  GENOTYPE
=====
52      61      GI      GACGTCAAGT TCCCGGGTGG CGGTCAGATC GTTGGTGGAG TTTACTTGTT GCCGCGCAGG
53      61      GACGTCAAGT TCCCGGGTGG CGGTCAGATC GTTGGTGGAG TTTACTTGTT GCCGCGCAGG
54      61      GACGTCAAGT TCCCGGGTGG CGGTCAGATC GTTGGTGGAG TTTACTTGTT GCCGCGCAGG
55      61      GACGTCAAGT TCCCGGGTGG CGGTCAGATC GTTGGTGGAG TTTACTTGTT GCCGCGCAGG
56      61      GACGTCAAGT TCCCGGGTGG CGGTCAGATC GTTGGTGGAG TTTACTTGTT GCCGCGCAGG
57      61      GACGTCAAGT TCCCGGGTGG CGGTCAGATC GTTGGTGGAG TTTACTTGTT GCCGCGCAGG
=====
58      61      GII     GACGTCAAGT TCCCGGGCGG TGGCCAGGTC GTTGGTGGAG TTTACTTGTT GCCGCGCAGG
59      61      GACGTCAAGT TCCCGGGCGG TGGTCAGATC GTTGGTGGAG TTTACTTGTT GCCGCGCAGG
60      61      GACGTCAAGT TCCCGGGCGG TGGTCAGATC GTTGGTGGAG TTTACTTGTT GCCGCGCAGG
61      61      GACGTCAAGT TCCCGGGCGG TGGTCAGATC GTTGGTGGAG TTTACTTGTT GCCGCGCAGG
62      61      GACGTCAAGT TCCCGGGCGG TGGTCAGATC GTTGGTGGAG TTTACTTGTT GCCGCGCAGG
63      61      GACGTCAAGT TCCCGGGCGG TGGTCAGATC GTTGGTGGAG TTTACTTGTT GCCGCGCAGG
64      61      GACGTCAAGT TCCCGGGCGG TGGTCAGATC GTTGGTGGAG TTTACTTGTT GCCGCGCAGG
=====
65      61      GIII    GACGTCAAGT TCCCGGGCGG TGGCCAGATC GTTGGCGGAG TATACCTGCT GCCGCGCAGG
66      61      GACGTCAAGT TCCCGGGCGG TGGTCAGATC GTTGGCGGAG TATACCTGCT GCCGCGCAGG
=====
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Fig. 5c

CORE REGION (3/9)

SEQUENCE			GENOTYPE		SEQUENCE	
ID NUMBER						
52	121	GI	GGCCCTAGAT	TGGGTGTGCG	CGCGACGAGA	AAGACTTCCG AGCGGTCGCA ACCTCGAGGT
53	121		GGCCCTAGAT	TGGGTGTGCG	CGCGACGAGG	AAGACTTCCG AGCGGTCGCA ACCTCGAGGT
54	121		GGCCCTAGAT	TGGGTGTGCG	CGCGACGAGG	AAGACTTCCG AGCGGTCGCA ACCTCGAGGT
55	121		GGCCCTAGAT	TGGGTGTGCG	CACGACGAGG	AAGACTTCCG AGCGGTCGCA ACCTCGAGGT
56	121		GGCCCTAGAT	TGGGTGTGCG	CGCGACGAGG	AAGACTTCCG AGCGGTCGCA ACCTCGAGGT
57	121		GGCCCTAGAT	TGGGTGTGCG	CGCGACGAGG	AAGACTTCCG AGCGGTCGCA ACCTCGTGGT
58	121	GII	GGCCCCAGGT	TGGGTGTGCG	CGCGACTAGG	AAGACTTCCG AGCGGTCGCA ACCTCGTGGA
59	121		GGCCCCAGGT	TGGGTGTGCG	CGCGACTAGG	AAGACTTCCG AGCGGTCGCA ACCTCGTGGA
60	121		GGCCCCAGGT	TGGGTGTGCG	CGCGACTAGG	AAGACTTCCG AGCGGTCGCA ACCTCGTGGA
61	121		GGCCCCAGGT	TGGGTGTGCG	CGCGACTAGG	AAGACTTCCG AGCGGTCGCA ACCTCGTGGA
62	121		GGCCCCAGGT	TGGGTGTGCG	CGCGACTAGG	AAGACTTCCG AGCGGTCGCA ACCTCGTGGA
63	121		GGCCCCAGGT	TGGGTGTGCG	CGCGACTAGG	AAGACTTCCG AGCGGTCGCA ACCTCGTGGA
64	121		GGCCCCAGGT	TGGGTGTGCG	CGCGACTAGG	AAGACTTCCG AGCGGTCGCA ACCTCGTGGA
65	121	GIII	GGCCCCAGAT	TGGGTGTGCG	CGCGACGAGG	AAACTTCCG AACGATCCCA GCCACGCGGA
66	121		GGCCCCAGGT	TGGGTGTGCG	CGCGACGAGG	AAACTTCCG AACGATCCCA GCCACGTTGG

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Fig. 5d

CORE REGION (4/9)

16/2/

SEQUENCE ID NUMBER	GENOTYPE	
52	GI	181 AGACGTCAGC CTATCCCCAA GGCTCGTCGG CCGAGGGCA GGACCTGGGC TCAGCCCGGG
53		181 AGACGTCAGC CTATCCCCAA GGCGGTCTGG CCGAGGGCA GGACCTGGGC TCAGCCCGGG
54		181 AGACGTCAGC CTATCCCTAA GGCGGTCTGG CCGAGGGCA GGACCTGGGC TCAGCCCGGG
55		181 AGACGTCAGC CCATCCCCAA GGCTCGTCGA CCGAGGGCA GGACCTGGGC TCAGCCCGGG
56		181 AGACGTCAGC CTATCCCCAA GGACGTCTGG CCGAGGGTA GGACCTGGGC TCAGCCCGGG
57		181 AGACGTCAGC CTATCCCCAA GGCGGTCTGG CCGAGGGCA GGACCTGGGC TCAGCCCGGG
58	GII	181 AGGCGACAAC CTATCCCCAA GGCTCGCCAG CCGAGGGCA GGCGCTGGGC TCAGCCCGGG
59		181 AGGCGACAAC CTATCCCCAA GGCTCGCCAG CCGAGGGCA GGCGCTGGGC TCAGCCCGGG
60		181 AGGCGACAAC CTATCCCCAA GGCTCGCCAG CCGAGGGCA GGCTCTGGGC TCAGCCCGGG
61		181 AGGCGACAAC CTATCCCCAA GGCTCGCCAG CCGAGGGTA GGCGCTGGGC TCAGCCCGGG
62		181 AGGCGACAAC CTATCCCCAA GGCTCGCCAG CCGAGGGCA GGCGCTGGGC TCAGCCCGGG
63		181 AGGCGACAAC CTATCCCCAA GGCTCGCCAG CCGAGGGCA GGCGCTGGGC TCAGCCCGGG
64		181 AGGCGACAAC CTATCCCCAA GGCTCGCCAG CCGAGGGCA GGCGCTGGGC TCAGCCCGGG
65	GIII	181 AGGCGTCAGC CCATCCCTAA AGATCGTCGC ACCGTGGCA AGTCCTGGG AAGGCCAGGA
66		181 AGGCGTCAGC CCACTCCCCA AGATCGTCGC ACCGTGGCA AGTCCTGGG GAAGCCAGGA

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Fig. 5e

CORE REGION (5/9)

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=====
SEQUENCE
ID NUMBER  GENOTYPE
=====
52      241      GI      TACCCCTTGGC CCCTCTATGG CAATGAGGGC TCGGGGTGGG CCGGATGGCT CCTGTCTCCC
53      241      TACCCCTTGGC CCCTCTATGG CAATGAGGGT TCGGGGTGGG CCGGATGGCT CCTGTCTCCC
54      241      TACCCCTTGGC CCCTCTATGG TAATGAGGGT TCGGGATGGG CCGGATGGCT CCTGTCTCCC
55      241      TACCCCTTGGC CCCTCTATGG CAATGAGGGC TCGGGGTGGG CCGGATGGCT CCTGTCTCCC
56      241      TACCCCTTGGC CCCTCTATGG CAATGAGGGT TCGGGGTGGG CCGGATGGCT CCTGTCTCCC
57      241      TACCCCTTGGC CCCTCTATGG CAATGAGGGT TCGGGGTGGG CCGGATGGCT CCTGTCTCCC
=====
58      241      GII     TACCCCTTGGC CCCTCTATGG CAATGAGGGT ATGGGGTGGG CAGGATGGCT CCTGTACACC
59      241      TACCCCTTGGC CCCTCTATGG CAACGAGGGT ATGGGGTGGG CAGGATGGCT CCTGTACACC
60      241      TAQCCCTTGGC CCCTCTATGG CAACGAGGGT ATGGGGTGGG CAGGATGGCT CCTGTACACC
61      241      TACCCCTTGGC CCCTCTATGG CAATGAGGGT ATGGGGTGGG CAGGATGGCT CCTGTACACC
62      241      TATCCCTTGGC CCCTCTATGG CAATGAGGGT CTGGGGTGGG CAGGATGGCT CCTGTACACC
63      241      TACCCCTTGGC CCCTCTATGG CAATGAGGGT ATGGGGTGGG CAGGATGGCT CCTGTACACC
64      241      TACCCCTTGGC CCCTCTATGG CAATGAGGGT ATGGGGTGGG CAGGATGGCT CCTGTACACC
=====
65      241      GIII    TATCCCTTGGC CCCTGTATGG GAATGAGGGT CTCGGCTGGG CAGGATGGCT CCTGTACACC
66      241      TACCCCTTGGC CCCTGTATGG GAATGAGGGT CTCGGCTGGG CAGGATGGCT CCTGTACACC
=====
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Fig. 5f

CORE REGION (6/9)

```
=====
SEQUENCE
ID NUMBER  GENOTYPE
=====
52      301  CGTGGCTCTC GGCCTAGCTG GGGCCCCACA GACCCCGCGC GTAGGTCGCG CAATTGGGT
53      301  CGTGGCTCTC GGCCTAGCTG GGGCCCCACA GACCCCGCGC GTAGGTCGCG CAATTGGGT
54      301  CGTGGCTCTC GGCCTAGCTG GGGCCCCACA GACCCCGCGC GTAGGTCGCG CAATTGGGT
55      301  CGTGGCTCTC GGCCTAGCTG GGGCCCCACA GACCCCGCGC GTAGGTCGCG CAATTGGGT
56      301  CGCGGCTCTC GGCCTAGCTG GGGCCCCACA GACCCCGCGC GTAGGTCGCG CAATTGGGT
57      301  CGTGGCTCTC GGCCTAGCTG GGGCCCCACA GACCCCGCGC GTAGGTCGCG CAATTGGGT
=====
58      301  CGTGGCTCTC GGCCTAGCTG GGGCCCCACA GACCCCGCGC GTAGGTCGCG TAATTGGGT
59      301  CGTGGCTCTC GGCCTAGCTG GGGCCCCACA GACCCCGCGC GTAGGTCGCG TAATTGGGT
60      301  CGCGGCTCCC GGCCTAGCTG GGGCCCCACA GACCCCGCGC GTAGGTCGCG TAATTGGGT
61      301  CGCGGCTCCC GGCCTAGCTG GGGCCCCACA GACCCCGCGC GTAGGTCGCG TAATTGGGT
62      301  CGCGGCTCTC GGCCTAGCTG GGGCCCCACA GACCCCGCGC GTAGGTCGCG CAACTGGGT
63      301  CGTGGTTCTC GGCCTAGCTG GGGCCCCACA GACCCCGCGC GTAGGTCGCG CAATTGGGT
64      301  CGCGGCTCCC GGCCTAGCTG GGGCCCCAAA GACCCCGCGC GTAGGTCGCG TAATTGGGT
=====
65      301  CGTGGCTCTC GCCCTTCATG GGGCCCCACT GACCCCGCGC ATAGATCAG CAACTGGGT
66      301  CGCGGTTCTC GCCCTTCATG GGGCCCCACT GACCCCGCGC ATAGATCAG CAACTGGGT
=====
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Fig. 5g

CORE REGION (7/9)

=====

SEQUENCE	ID NUMBER	GENOTYPE
52	361	GI
53	361	
54	361	
55	361	
56	361	
57	361	
58	361	GII
59	361	
60	361	
61	361	
62	361	
63	361	
64	361	
65	361	GIII
66	361	

=====

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Fig. 5h

CORE REGION (8/9)

20/21

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=====
SEQUENCE
ID NUMBER  GENOTYPE
=====
52  421  G1  GGC GCCCTC TTGAGGCG TGCCAGGCC CTGGCGCATG GCGTCCGGT TCTGGAAGAC
53  421  GGC GCCCTC TTGAGGCG TGCCAGGGT CTGGCGCATG GCGTCCGGT TCTGGAAGAC
54  421  GGC GCCCTC TTGAGGCG TGCCAGGCC CTGGCGCATG GCGTCCGGT TCTGGAAGAC
55  421  GGC GCCCTC TTGAGGCG TGCCAGAGCC CTGGCGCATG GCGTCCGGT TCTGGAAGAC
56  421  GGC GCCCTC TTGAGGCG TGCCAGGCC CTGGCGCATG GCGTCCGGT TCTGGAAGAC
57  421  GGC GCCCTC TTGAGGCG TGCCAGGCC CTGGCGCATG GCGTCCGGT TCTGGAAGAC
=====
58  421  G11  GGC GCCCCC TTAGGGCG TGCCAGGCC TTGGCGCATG GCGTCCGGT TCTGGAAGAC
59  421  GGC GCCCCC TAGGGGCG TGCCAGGCC CTGGCGCATG GTGTCCGGT TCTGGAAGAC
60  421  GGC GCCCCC TAGGGGCG TGCCAGGCC CTGGCGCATG GTGTCCGGT TCTGGAAGAC
61  421  GGC GCCCCC TAGGGGCG TGCCAGGCC CTGGCGCATG GCGTCCGGT TCTGGAAGAC
62  421  GGC GCCCCC TTAGGGCG TGCCAGGCC CTGGCGCATG GCGTCCGGT TCTGGAAGAC
63  421  GGC GCCCCC TAGGGGCG TGCCAGGCC CTGGCGCATG GCGTCCGGT TCTGGAAGAC
64  421  GGC GCCCCT TAGGGGCG TGCCAGGCC CTGGCGCATG GCGTCCGGT TCTGGAAGAC
=====
65  421  G111 GGC GCCCCG TTGAGGCG TGCCAGAGT CTCGCCACG GAGTGAGGT TCTGGAAGAT
66  421  GGTGCCCG TTGTGTGT CGCCAGAGC CTGGCCCATG GGTGAGGT TCTGGAAGAC
=====
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Fig. 5i

CORE REGION (9/9)

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=====
SEQUENCE
ID NUMBER GENOTYPE
=====
52      481      GI      GCGGTGAAC T ATGCAACAGG GAACCTTCCT GGTGGCTCTT TCTCTATCTT CCTCTGGCC CTGCTCTCT
53      481      GCGGTGAAC T ATGCAACAGG GAACCTTCCT GGTGGCTCTT TCTCTATCTT CCTCTGGCC CTGCTCTCT
54      481      GCGGTGAAC T ATGCAACAGG GAATCTTCCT GGTGGCTCTT TCTCTATCTT CCTCTGGCC CTGCTCTCT
55      481      GCGGTGAAC T ATGCAACAGG GAACCTTCCT GGTGGCTCTT TCTCTATCTT CCTCTGGCC CTGCTCTCT
56      481      GCGGTGAAC T ATGCAACAGG GAACCTTCCT GGTGGCTCTT TCTCTATCTT CCTCTGGCC CTGCTCTCT
57      481      GCGGTGAAC T ATGCAACAGG GAACCTTCCT GGTGGCTCTT TCTCTATCTT CCTCTGGCC CTGCTCTCT
=====
58      481      GII      GCGGTGAAC T ATGCAACAGG GAATCTGCCC GGTGGCTCTT TCTCTATCTT CCTCTGGCT CTGCTGTCC
59      481      GCGGTGAAC T ATGCAACAGG GAATTTGCCC GGTGGCTCTT TCTCTATCTT CCTCTGGCT CTGCTGTCC
60      481      GCGGTGAAC T ATGCAACAGG GAATTTGCTT GGTGGCTCTT TCTCTATCTT CCTCTGGCT CTGCTGTCC
61      481      GCGGTGAAC T ATGCAACAGG GAATCTGCCC GGTGGCTCTT TCTCTATCTT CCTCTGGCT TTGCTGTCC
62      481      GCGGTGAAC T ATGCAACAGG GAATTTGCCC GGTGGCTCTT TCTCTATCTT CCTCTGGCT TTGCTGTCC
63      481      GCGGTGAAC T ATGCAACAGG GAATCTGCCC GGTGGCTCTT TCTCTATCTT CCTCTGGCT TTGCTGTCC
64      481      GCGGTGAAC T ATGCAACAGG GAATCTACCC GGTGGCTCTT TCTCTATCTT CCTCTGGCT TTGCTGTCC
=====
65      481      GIII     GGGGTAAAT T ATGCAACAGG GAATTTGCCC GGTGGCTCTT TCTCTATCTT TCTCTAGCC CTCTTGTCT
66      481      GGGATAAAT T ATGCAACAGG GAATCTGCCC
=====
549 Total
=====
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